Ovarian Cancer: Prevention and Detection of the Disease and its Recurrence
Molecular Targets and the Immune System

Ronald B. Herberman Conference Center
Second Floor, UPMC Cancer Pavilion
5150 Centre Avenue
Pittsburgh, PA 15232

Monday, October 24, 2005
and
Tuesday, October 25, 2005

SPONSORED BY:

University of Pittsburgh School of Medicine
Center for Continuing Education in the Health Sciences
In collaboration with the Marsha Rivkin Center for Ovarian Cancer Research

FINANCIAL SUPPORT FOR THIS EVENT WAS PROVIDED BY:

GSK Oncology • Unither Pharmaceuticals, Inc.
CIPHERGEN Diagnostics • Douglas Laboratories
Gynecologic Oncology Group (GOG) Tissue Bank
MedImmune Oncology, Inc • Merck & Co, Inc. • MGI Pharma, Inc.
Precision Therapeutics, Inc. • Roche Oncology
Ovarian Cancer: Prevention and Detection of the Disease and its Recurrence
Molecular Targets and the Immune System

Monday, October 24 and Tuesday, October 25, 2005

Overview and Objectives

The purpose of this international symposium is to bring together experts in ovarian cancer research, clinicians, public health policy, and consumer advocates to discuss the scientific and health implications of prevention, screening, early detection and treatment modalities for ovarian cancer as well as detection, prevention and treatment modalities for disease recurrence. Topics will be covered in general, although special emphasis will be given in each session to molecular targets and the immune system. In addition to the scientific presentations, there will be a special breakout session for and by consumer advocates.

At the conclusion of the symposium, participants should:
1. Have a greater understanding of the underlying molecular, biologic and genetic mechanisms involved in ovarian cancer development and how these mechanisms can be targets for prevention and detection of the disease and its recurrence
2. Be familiar with emerging chemo-prevention and recurrence prevention agents and approaches aimed at specific molecular and biologic targets
3. Be informed about new high-throughput technologies and their application to ovarian cancer research
4. Understand the impact of the disease on the well-being of women and their families
5. Identify new areas of research based on the molecular mechanisms of the disease

Who Should Attend

Participation by all individuals is encouraged, especially ovarian cancer researchers, clinicians, policy makers, and consumer advocates, including ovarian cancer survivors and their families.

Continuing Education Credit

The University of Pittsburgh School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

The University of Pittsburgh School of Medicine designates this educational activity for a maximum of 13.5 Category 1 credits toward the AMA Physician's Recognition Award. Each physician should claim only those credits that he/she actually spent in the educational activity.

Other health care professionals are awarded 1.35 continuing education units (CEU's) which are equal to 13.5 contact hours.
Program: Monday, October 24, 2005

7:30 AM Opening Key Note
  Jeff Boyd, PhD

Epidemiology of Ovarian Cancer
8:00 AM Molecular Studies of Ovarian Cancer in a Multiethnic Population
  Marc T Goodman, PhD, MPH
8:25 AM Screening for Ovarian Cancer in High Risk Women
  Patricia Hartge, ScD
8:50 AM New Directions in Ovarian Cancer Epidemiology and Prevention
  Joellen Schildkraut, PhD
9:15 AM Panel Discussion
  Roberta B Ness, MD, MPH
9:30 AM Break

From Epidemiology to Ovarian Cancer Biology
10:00 AM Overview
  Emanuela Taioli, MD, PhD
10:05 AM Pregnancy and Ovarian Cancer: Role of Endogenous Hormones
  Paolo Toniolo, MD, MSPH
10:30 AM Modeling Epithelial Ovarian Cancer in the Mouse
  Denise Connolly, PhD
10:55 AM Mechanism of Ovarian Cancer Predisposition in BRCA1 Mutation Carriers - Implications for Ovarian Cancer Screening and Prevention
  Louis Dubeau, MD, PhD
11:20 AM Discussion
  Emanuela Taioli, MD, PhD
11:35 AM Lunch

Screening and Early Detection - Emerging Technologies
12:45 PM Overview
  Joel Weissfeld, MD, MPH
12:50 PM A Proteomics Approach to the Detection of Ovarian Cancer
  Eric Fung, MD, PhD
1:15 PM Application of Proteomics Technologies to Advance our Understanding of Ovarian Cancer
  Elise Kohn, MD
1:40 PM The Detection of Early Stage Ovarian Cancer - Is this a Clinical Reality?
  David Fishman, MD
2:05 PM Antigen-based Technology for Screening and Detecting Ovarian Cancer and its Recurrence
  Anna Lokshin, PhD
2:30 PM Panel Discussion
  Joel Weissfeld, MD, MPH
2:45 PM Break

Genetics and Epigenetics
3:15 PM Overview
  Francesmary Modugno, PhD, MPH
3:20 PM An Update on the HapMap
  Wendy Wang, PhD
3:45 PM Prevention and Early Detection in Women at Increased Genetic Risk
  Mark H Greene, MD
4:10 PM Variable Expression and Activity of Pharmacokinetic Variables in Ovarian Tumors
  Julie A DeLoia, PhD
4:35 PM Panel Discussion
  Francesmary Modugno, PhD, MPH

Reception and Poster Session
5:00 to 7:30 PM: Reception and Poster Session (Herberman Conf Ctr Room 201AB)
6:00 to 7:00 PM: Mini-Symposium Presentations (Herberman Conf Ctr Auditorium)
  Chair: Julie A DeLoia, PhD
### Program: Tuesday, October 25, 2005

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 AM</td>
<td>Opening Plenary&lt;br&gt;Karen Johnson, MD, PhD, MPH</td>
</tr>
<tr>
<td>8:30 AM</td>
<td>Beyond Treating the Patient - Survivorship Issues&lt;br&gt;Julene Fabrizio &amp; Patricia Goldman</td>
</tr>
<tr>
<td>9:00 AM</td>
<td>Ovarian Cancer: Insider Perspectives&lt;br&gt;Barbara Smith &amp; Cynthia DePastino</td>
</tr>
<tr>
<td>9:05 AM</td>
<td>Psycho-social Issues in Diagnosis and Recurrence: Effects on Patients and Their Families&lt;br&gt;Heidi Donovan, PhD</td>
</tr>
<tr>
<td>9:30 AM</td>
<td>Integrating Complementary Medicine with Conventional Treatment&lt;br&gt;Maria B Yaramus, PharmD</td>
</tr>
<tr>
<td>9:55 AM</td>
<td>Panel Discussion&lt;br&gt;Francesmary Modugno, PhD, MPH</td>
</tr>
<tr>
<td>10:10 AM</td>
<td>Break</td>
</tr>
<tr>
<td>10:30 AM</td>
<td>New Directions in Therapeutics and Prevention I</td>
</tr>
<tr>
<td>10:35 AM</td>
<td>Overview&lt;br&gt;Kristen Zorn, MD</td>
</tr>
<tr>
<td>10:40 AM</td>
<td>Peritoneal Immunotherapy&lt;br&gt;Ralph S Freedman, MD, PhD</td>
</tr>
<tr>
<td>11:00 AM</td>
<td>Vaccines Targeting Ovarian Cancer&lt;br&gt;Mary (Nora) Disis, MD</td>
</tr>
<tr>
<td>11:25 AM</td>
<td>Immunobiology of MUC1 Tumor Antigen: Lessons Learned and Future Implications in Ovarian Cancer&lt;br&gt;Anda Vlad, MD, PhD</td>
</tr>
<tr>
<td>11:50 AM</td>
<td>Evolution of NY-ESO-1 Vaccine Therapy for Ovarian Cancer&lt;br&gt;Robert P Edwards, MD</td>
</tr>
<tr>
<td>12:15 PM</td>
<td>Panel Discussion&lt;br&gt;Kristen Zorn, MD</td>
</tr>
<tr>
<td>12:30 PM</td>
<td>Lunch</td>
</tr>
<tr>
<td>1:30 PM</td>
<td>New Directions in Therapeutics and Prevention II</td>
</tr>
<tr>
<td>1:35 PM</td>
<td>Overview&lt;br&gt;Thomas Krivak, MD</td>
</tr>
<tr>
<td>1:40 PM</td>
<td>Inflammatory Modulation of Cancer&lt;br&gt;Thomas Rutherford, MD, PhD</td>
</tr>
<tr>
<td>2:00 PM</td>
<td>Oregovomab: Challenges, Lessons, &amp; Opportunities&lt;br&gt;Christopher Nicodemus, MD</td>
</tr>
<tr>
<td>2:25 PM</td>
<td>Novel Approaches for Platinum Refractive Cancer&lt;br&gt;Robert P Edwards, MD</td>
</tr>
<tr>
<td>2:50 PM</td>
<td>Individualizing Cancer Therapy: Current Status, Future Directions&lt;br&gt;Holly Gallion, MD</td>
</tr>
<tr>
<td>3:15 PM</td>
<td>Panel Discussion&lt;br&gt;Thomas Krivak, MD</td>
</tr>
<tr>
<td>3:30 PM</td>
<td>Concluding Remarks&lt;br&gt;Francesmary Modugno, PhD, MPH</td>
</tr>
</tbody>
</table>
FACULTY LISTING

Symposium Director
Francesmary Modugno, PhD, MPH • Assistant Professor, Department of Epidemiology, University of Pittsburgh Graduate School of Public Health and University of Pittsburgh Cancer Institute • Pittsburgh, Pennsylvania

Guest Faculty
Jeff Boyd, PhD • Member and Attending Biologist, Departments of Surgery and Medicine, Memorial Sloan-Kettering Cancer Center • New York, New York
Denise Connolly, PhD • Assistant Member, Medical Sciences, Fox Chase Cancer Center • Philadelphia, Pennsylvania
Cynthia DePastino • Survivor and Vice President, National Ovarian Cancer Coalition Pittsburgh Division • Pittsburgh, Pennsylvania
Mary L (Nora) Disis, MD • Associate Professor, Division of Oncology, University of Washington • Seattle, Washington
Louis Dubeau, MD, PhD • Professor, Department of Pathology, University of Southern California Keck School of Medicine and USC/Norris Comprehensive Cancer Center • Los Angeles, California
Julene Fabrizio • President, National Ovarian Cancer Coalition • Boca Raton, Florida
David Fishman, MD • Professor and Director, Gynecologic Oncology, Cancer Prevention and Early Detection, New York University Cancer Institute • New York, New York
Ralph Freedman • Professor, Department of Gynecologic Oncology, The University of Texas M. D. Anderson Cancer Center • Houston, Texas
Eric Fung, MD, PhD • Vice President of Clinical Affairs, Ciphergen Diagnostics • Fremont, California
Holly Gallion, MD • Vice President, Precision Therapeutics • Pittsburgh, Pennsylvania
Patricia Goldman • President Emeritus, Ovarian Cancer National Alliance • Washington, District of Columbia
Marc T Goodman, PhD, MPH • Professor (Researcher), Cancer Research Center of Hawaii; Professor of Public Health; Graduate Faculty in the Interdisciplinary Biomedical Sciences Graduate Program, University of Hawaii • Honolulu, Hawaii
Mark H Greene, MD • Chief, Clinical Genetics Branch, National Cancer Institute • Rockville, Maryland
Patricia Hartge, ScD • Deputy Director, Epidemiology and Biostatistics Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute • Rockville, Maryland
Karen A Johnson, MD, PhD, MPH • Chief, Breast and Gynecologic Cancer Research Group, Division of Cancer Prevention, National Cancer Institute • Rockville, Maryland
Elise Kohn, MD • Senior Investigator and Section Chief, Molecular Signaling Section, Laboratory of Pathology and Co-Chair, Breast and Gynecologic Malignancies Faculty, Center for Cancer Research, National Cancer Institute • Bethesda, Maryland
Christopher Nicodemus, MD • Senior Vice President, Clinical Research & Development, Unither Pharmaceuticals, Inc • Wellesley, Massachusetts
Kunle Odunsi, MD, PhD • Assistant Professor, Department of Obstetrics & Gynecology, School of Medicine & Biomedical Sciences, University at Buffalo • Buffalo, New York
Thomas Rutherford, MD, PhD • Associate Professor, Yale University School of Medicine • New Haven, Connecticut
Joellen Schildkraut, PhD • Associate Professor, Community and Family Medicine, Program of Cancer Prevention, Detection and Control Research, Duke University • Durham, North Carolina
Barbara Smith • Ovarian Cancer Family Member and Secretary, National Ovarian Cancer Coalition Pittsburgh Division • Pittsburgh, Pennsylvania
Paolo Toniolo, MD, MSPH • Professor, Department of Epidemiology, New York University School of Medicine • New York, New York
Wendy Wang, PhD • Program Director, Cancer Biomarkers Research Group, Division of Cancer Prevention, National Cancer Institute • Rockville, Maryland
University of Pittsburgh/University of Pittsburgh Medical Center Faculty

Julie DeLoia, PhD • Associate Professor, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh and Magee-Womens Research Institute • Pittsburgh, Pennsylvania

Heidi Donovan, PhD • Assistant Professor, Department of Acute/Tertiary Care, University of Pittsburgh School of Nursing • Pittsburgh, Pennsylvania

Robert P Edwards, MD • Professor, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh and Magee-Womens Research Institute • Pittsburgh, Pennsylvania

Joseph L Kelley, MD • Associate Professor, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh and Magee-Womens Research Institute • Pittsburgh, Pennsylvania

Thomas Krivak, MD • Assistant Professor, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh and Magee-Womens Research Institute • Pittsburgh, Pennsylvania

Anna Lokshin, PhD • Associate Professor, University of Pittsburgh School of Medicine • Pittsburgh, Pennsylvania

Roberta B Ness, MD, MPH • Professor and Chair, Department of Epidemiology, University of Pittsburgh Graduate School of Public Health and University of Pittsburgh Cancer Institute • Pittsburgh, Pennsylvania

Emanuela Taioli, MD, PhD • Professor, Department of Epidemiology, University of Pittsburgh Graduate School of Public Health and University of Pittsburgh Cancer Institute • Pittsburgh, Pennsylvania

Anda Vlad, MD, PhD • Research Assistant Professor, Department of Immunology, University of Pittsburgh School of Medicine • Pittsburgh, Pennsylvania

Joel Weissfeld, MD, MPH • Associate Professor, Department of Epidemiology, University of Pittsburgh Graduate School of Public Health and University of Pittsburgh Cancer Institute • Pittsburgh, Pennsylvania

Maria B Yaramus, PharmD • Assistant Professor, Department of Pharmacy and Therapeutics, University of Pittsburgh School of Pharmacy • Pittsburgh, Pennsylvania

Kristin Zorn, MD • Assistant Professor, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh and Magee-Womens Research Institute • Pittsburgh, Pennsylvania

Symposium Program Committee Members

Francesmary Modugno, PhD, MPH, Chair

Julie A DeLoia, PhD

Robert P Edwards, MD

Joseph L Kelley, MD

Roberta B Ness, MD, MPH

Maria B Yaramus, PharmD

Special Acknowledgement and Thanks

The Symposium Director and Program Committee would like to acknowledge and thank the following individuals for their efforts and help during the planning and implementation of this symposium:

- Chandra Marriott, MPH, Symposium Coordinator
- Bernard Goldstein, MD, Dean, Graduate School of Public Health
- Ronald Herberman, MD, Director, University of Pittsburgh Cancer Institute

Jessica Albano

Glenn Allen

Aab Arnold

Brian Balich

Tricina Cash

Clare Collins

Carey Cyphert

Amy Ditta

Keith Durst

Jeffrey Eppinger

Gretchen Gierach

Kerry Harrity

Clover Hoffacker

Alana Hudson

Shari Hutchison

Betty Kotowski

Claudia Lieras

Laura Markowitz

Kambra McConnel

Jocelyn Moore

John Palko

Amy Phillips

Katherine Reeves

Joanne Rooney

Tracy Salerno

Cheryl Schmitt

Katherine Simpson

Bob Stoeckle

Jim Swyers

Robert Williams

Mary Yagjian

Mark Yobbi
FACULTY DISCLOSURE

Faculty for this activity have been required to disclose all relationships with any proprietary entity producing health care goods or services, with the exemption of non-profit or government organizations and non-health care related companies.

No significant financial relationships with commercial entities were disclosed by:

Symposium Faculty
Denise C Connolly, PhD
Julie DeLoia, PhD
Cynthia DePastino
Heidi Donovan, PhD
Louis Dubeau, MD, PhD
Robert P Edwards, MD
Julene Fabrizio
David Fishman, MD
Ralph S Freedman, MD, PhD
Patricia Goldman

Symposium Faculty
Marc T Goodman, PhD, MPH
Mark H Greene, MD
Patricia Hartge, ScD
Karen A Johnson, MD, PhD, MPH
Joseph L Kelley, MD
Elise C Kohn, MD
Thomas Krivak, MD
Anna Lokshin, PhD
Francesmary Modugno, PhD, MPH
Robert B Ness, MD, MPH

Joellen Schildkraut, PhD
Barbara Smith
Emanuela Taioli, MD, PhD
Paolo Toniolo, MD, MSPH
Anda Vlad, MD, PhD
Wendy Wang, PhD
Joel Weissfeld, MD, MPH
Maria B Yaramus, PharmD
Kristin Zorn, MD

Mini-Symposium Abstract Authors
Giovanni Aletti
Christine Ambrosone
Dimcho Bachvarov
Magdalena Bachvarova
Jonathan Berek
Keith Bible
Jessica Chan
Jeremy Chien
William Cliby
Richard DiCioccio
Simon Gayther
Lynn Hartmann
Estrid Hogdall
Claus Hogdall

Alan Hutson
Ian Jacobs
Scott Kaufmann
Susanne Kruger Kjaer
Shashikant Lele
Sylvain L'Esperance
Susan McCann
Usha Menon on behalf of the
UKCTOCS Group
Gregory Miller
Kunle Odunsi
Paul Pharaoah
Bruce Ponder
Ion Popa

Feng Qian
Lydia Quaye
Susan Ramus
JianYu Rao
David Seligson
Thomas Sellers
Ravi Shridhar
Viji Shridhar
Peggy Soung
Jonathan Tammela
Bernard Tetu
Alice Whitemore
Robert Wollman

The following information was disclosed:

Symposium Faculty
Jeff Boyd, PhD: Stockholder in Genentech.
Mary L Disis, MD: Grant/research support from 3M; Consultant to Dendreon, Merck & Co, Inc, and Protiva Biotherapeutics.
Eric Fung, MD, PhD: Stockholder in Ciphergen Diagnostics.
Holly Gallion, MD: Stockholder in Precision Therapeutics.
Christopher F Nicodemus, MD, FACP: Stockholder and employee of Unither Pharmaceuticals, Inc.
Kunle Odunsi, MD, PhD: Grant/research support from Aventis Pharmaceuticals.
Thomas Rutherford, MD, PhD: Grant/research support from Precision Therapeutics.

Mini-Symposium Abstract Authors
Robert M Crowl, PhD: Retiree from Novartis Pharmaceuticals.
Honglin Song: Grant/research support from Well Being/University of Cambridge, UK.
John P Geisler: Grant/research support from Women’s Oncology Research and Davenport Foundation; Consultant to Tibotec Therapeutics.

* No disclosure form was obtained from deceased abstract author James Alderfer.
ACKNOWLEDGEMENT OF COMMERCIAL & OTHER SUPPORT

We gratefully acknowledge the following organizations for their support of this activity:

National Cancer Institute

U.S. Department of Health and Human Services, Office on Women's Health

National Ovarian Cancer Coalition

Tibotec Therapeutics

LHAS

sanofi aventis

GSK Oncology

Unither Pharmaceuticals, Inc

Ciphergen Diagnostics

Douglas Laboratories

MGI Pharma, Inc

MedImmune Oncology, Inc

Merck & Co, Inc

Gynecologic Oncology Group (GOG) Tissue Bank

Precision Therapeutics, Inc

Roche Oncology

DISCLAIMER STATEMENT

The information presented at this CME program represents the views and opinions of the individual presenters, and does not constitute the opinion or endorsement of, or promotion by, the UPMC Center for Continuing Education in the Health Sciences, UPMC / University of Pittsburgh Medical Center or Affiliates and University of Pittsburgh School of Medicine. Reasonable efforts have been taken intending for educational subject matter to be presented in a balanced, unbiased fashion and in compliance with regulatory requirements. However, each program attendee must always use his/her own personal and professional judgment when considering further application of this information, particularly as it may relate to patient diagnostic or treatment decisions including, without limitation, FDA-approved uses and any off-label uses.
Scientific Sessions
Genetic Analysis of Ovarian Cancer Histogenesis

Jeff Boyd, Ph.D.
Departments of Surgery and Medicine
Memorial Sloan-Kettering Cancer Center
New York, NY

Options for Reduction in Mortality from Ovarian Cancer

• Prevention
• Early detection
• Improved therapy

WHO Criteria for Disease Screening Program

• Important health problem
• Accepted treatment
• Diagnosis and treatment facilities available
• Suitable test or examination
• Recognizable latent or early symptomatic stage

Study Objectives

• What is the histologic origin of ovarian carcinoma?
• Is there an intermediate precursor lesion for ovarian carcinoma?
Where does ovarian cancer arise?

- Surface epithelium
- Morphologic alterations of surface epithelium
  - inclusion cyst
  - invagination
  - papillation
  - pseudostratification
- Secondary Müllerian system

Components of Secondary Müllerian System

- Paraovarian/paratubal cysts
- Rete ovarii
- Endosalpingiosis
- Endometriosis
- Endomucinosis

Dubeau, Gynecol Oncol 1999

Hypothetical Model of Ovarian Tumorigenesis

Drakpin and Hecht, Women’s Oncol Rev 2002

Epithelial Ovarian Tumors vs. Ovarian and Peritoneal Mesothelium

Urogenital Ridge (7 weeks)
Study Objectives

- What is the histologic origin of ovarian carcinoma?
- Is there an intermediate precursor lesion for ovarian carcinoma?

Is there an intermediate precursor lesion for ovarian carcinoma?

- Benign tumors (cystic adenomas)
- Borderline (low malignant potential) tumors
- Dysplasia
- Carcinoma in situ
- Hyperplasia
- "De novo"

Can genetic analysis be combined with morphologic analysis to gain insight into the early natural history of ovarian carcinoma?

Genetic Causes of Hereditary Susceptibility to Ovarian Cancer

- Sporadic (90%)
- BRCA1 (60%)
- BRCA2 (30%)
- HNPCC (5%)
- Other single genes (5%)

All Cancers are Genetic

“Hereditary” Cancer:

Birth → BRCA → Mut 1 Inherited → Mut 2 Somatic → Mut 3 Somatic → Mut 4 Somatic → Mut 5 Somatic → Mut 6 Somatic → Cancer

“Sporadic” Cancer:

Birth → Mut 1 Somatic → Mut 2 Somatic → Mut 3 Somatic → Mut 4 Somatic → Mut 5 Somatic → Mut 6 Somatic → Cancer

Minimal Molecular Genetic Requirements for BRCA-Linked Ovarian Tumorigenesis

- Inheritance of mutant BRCA allele through germline
- Somatic loss of wild-type BRCA allele
- Somatic mutational inactivation of TP53 tumor suppressor gene
Minimal Molecular Genetic Requirements for BRCA-Linked Ovarian Tumorigenesis

- Inheritance of mutant BRCA allele through germline
- Somatic loss of wild-type BRCA allele
- Somatic mutational inactivation of TP53 tumor suppressor gene

Chromosomal Mechanisms for TSG Recessivity

Germline

Initiated cell

Tumor

- Mitotic recombination
- Nondisjunction, loss
- Nondisjunction, reduplication

1. Hereditary
2. Sporadic

Somatic mutation

Immunohistochemical Analysis of p53 Expression in Ovarian Tissues from BRCA Heterozygotes

- Ovarian tissues removed prophylactically from 37 patients with a deleterious germline BRCA mutation
- Focal p53 expression observed in 10 (27%) of these specimens
- Invariably confined to morphologic alterations, such as cortical clefts and inclusion cysts
- Rarely observed in surface epithelium or non-epithelial components of ovary
- In most cases, confined to a single locus of epithelium

p53 Immunoreactivity in Ovarian Cystic Epithelium

- Growth arrest
- Apoptosis
- Angiogenesis inhibition
p53 Immunoreactivity in Ovarian Cystic Epithelium

Automated Sequence Analysis of TP53 Mutation in DNA from Microdissected Ovarian Epithelium

Detection of Mutant and Wild-type BRCA Alleles

Loss of Wild-type BRCA Allele in DNA from Microdissected Ovarian Epithelium
### Genetic Evidence of Tumorigenic Progression in Ovarian Epithelium from BRCA Heterozygotes

<table>
<thead>
<tr>
<th>Specimen #</th>
<th>BRCA Mutation</th>
<th>LOH</th>
<th>TP53 Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO3</td>
<td>BRCA1</td>
<td>Yes</td>
<td>H193R (C(\rightarrow)T)</td>
</tr>
<tr>
<td></td>
<td>4050del4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PO49</td>
<td>BRCA1</td>
<td>No</td>
<td>H179R (C(\rightarrow)T)</td>
</tr>
<tr>
<td></td>
<td>5382insC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PO67</td>
<td>BRCA2</td>
<td>Yes</td>
<td>S185N (A(\rightarrow)C)</td>
</tr>
<tr>
<td></td>
<td>6174delT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Stage I Ovarian Carcinomas from BRCA Heterozygotes

<table>
<thead>
<tr>
<th>Case</th>
<th>Stage</th>
<th>Histology</th>
<th>Grade</th>
<th>BRCA</th>
<th>TP53</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC3</td>
<td>IA</td>
<td>Em</td>
<td>3</td>
<td>5382insC</td>
<td>IVS8+1</td>
</tr>
<tr>
<td>OC6</td>
<td>IB</td>
<td>PS</td>
<td>2</td>
<td>185delAG</td>
<td>H179R</td>
</tr>
<tr>
<td>OC7</td>
<td>IC</td>
<td>PS</td>
<td>2</td>
<td>5382insC</td>
<td>C275Y</td>
</tr>
<tr>
<td>OC15</td>
<td>IC</td>
<td>CC</td>
<td>3</td>
<td>185delAG</td>
<td>N239insT</td>
</tr>
<tr>
<td>OC16</td>
<td>IC</td>
<td>Em</td>
<td>2</td>
<td>185delAG</td>
<td>R282W</td>
</tr>
</tbody>
</table>

### Dysplasia

- Cellular pleomorphism
- Nuclear atypia
- Loss of cellular architectural organization
- No evidence of stromal invasion
Stage I Ovarian Carcinomas from BRCA Heterozygotes

<table>
<thead>
<tr>
<th>Case</th>
<th>Tumor BRCA</th>
<th>Tumor TP53</th>
<th>Dysplasia BRCA</th>
<th>Dysplasia TP53</th>
<th>Normal BRCA</th>
<th>Normal TP53</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC3</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>OC6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>OC7</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>OC15</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>OC16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Relevance to sporadic ovarian carcinoma?

Immunohistochemical Analysis of p53 Expression in Normal Ovarian Tissues

- Ovarian tissues removed from 20 patients with benign disease not affecting ovaries
- Focal p53 expression observed in 5 (25%) of these specimens
- Invariably confined to morphologic alterations, such as cortical cleft (n = 1) and inclusion cysts (n = 4)
- Not observed in surface epithelium or non-epithelial components of ovary
- In all cases, confined to a single locus of epithelium

Stage I/II Sporadic Ovarian Carcinomas

- 145 stage I/II ovarian cancers from 20-yr period
- 23 cases with epithelial transition identified
  - 21 (91%) cases: inclusion cyst
  - 1 (4%) case: surface invagination
  - 1 (4%) case: surface epithelium
- In all 23 cases, noninvasive epithelial component consisted of normal epithelium and dysplasia adjacent to carcinoma
- TP53/p53 analysis
  - Normal and dysplastic cells p53 immunopositive in 23 (100%) cases
  - TP53 mutation evident in 11/23 (48%) invasive cancers
  - 2 cases with same mutation present in normal and dysplastic epithelium

What is the molecular phenotype of ovarian cyst vs. surface epithelium?
Expression Profiling of Cyst vs. Surface Epithelium in Clinically and Pathologically Normal Ovaries vs. Tumor

- LCM of cyst and surface epithelial cells from 18 normal ovaries; equal number of invasive, high grade, serous ovarian cancers
- Approximately 10,000 cells in each group, divided into three independent samples
- RNA subjected to three rounds of linear amplification
- Gene expression profiling using Affymetrix U133A oligonucleotide microarray (22,000 genes)

Expression Profiling of Cyst and Tumor vs. Surface Epithelium

- >1,000 genes differentially expressed between surface and cyst
- 657 genes differentially expressed in cyst and tumor compared to surface (418 up, 239 down)
  - Many oncogenic factors up
  - Many tumor suppressive factors down
- 276 genes differentially expressed in surface and tumor compared to cyst (88 up, 188 down)
  - Very few noteworthy genes with respect to plausible connection to neoplasia

Validation of Genes Differentially Expressed in Cyst and Tumor Vs. Surface Epithelium

- Schummer, Gene 1999
- Wang, Gene 1999
- Hough, Cancer Res 2000
- Ono, Cancer Res 2000
- Giordano, Am J Pathol 2001
- Welsh, Proc Natl Acad Sci USA 2001
- Wong, Biotechniques 2001
- Jazaeri, J Natl Cancer Inst 2002
- Zorn, Clin Cancer Res 2003
- Jazaeri, Mol Carcinog 2003
Validation of Genes Differentially Expressed in Cyst and Tumor Vs. Surface Epithelium

- Of 292 most significant known genes differentially expressed, 71 (24%) previously found differentially expressed in tumor vs. normal control in at least one of 10 published studies (using multiple platforms/approaches)
  - 37/186 (20%) up-regulated
  - 34/106 (32%) down-regulated
- 15 of 292 (5%) genes previously identified in at least two previous studies
  - TACSTD1: Tumor-associated calcium signal transducer 1 (five previous studies)
  - WFDC2: Whey-acidic protein type, four-disulfide core domain 2, putative ovarian cancer marker, HE4 (six previous studies)

<table>
<thead>
<tr>
<th>IHC</th>
<th>Surface Epithelium</th>
<th>Cyst Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>43 (91%)</td>
<td>35 (65%)</td>
</tr>
<tr>
<td>Positive</td>
<td>4 (9%)</td>
<td>19 (35%)</td>
</tr>
<tr>
<td>Total</td>
<td>47 (100%)</td>
<td>54 (100%)</td>
</tr>
</tbody>
</table>

FOS expression in ovarian surface and cystic epithelium

<table>
<thead>
<tr>
<th>IHC</th>
<th>Surface Epithelium</th>
<th>Cyst Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>12 (28%)</td>
<td>52 (90%)</td>
</tr>
<tr>
<td>Strong</td>
<td>35 (74%)</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>Total</td>
<td>47 (100%)</td>
<td>56 (100%)</td>
</tr>
</tbody>
</table>

\( P = 0.001 \)

TOP2A expression in ovarian surface and cystic epithelium

<table>
<thead>
<tr>
<th>IHC</th>
<th>Surface Epithelium</th>
<th>Cyst Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>43 (91%)</td>
<td>35 (65%)</td>
</tr>
<tr>
<td>Positive</td>
<td>4 (9%)</td>
<td>19 (35%)</td>
</tr>
<tr>
<td>Total</td>
<td>47 (100%)</td>
<td>54 (100%)</td>
</tr>
</tbody>
</table>

\( P = 0.002 \)

How to get from gene list(s) to biological insights?

Expression Analysis Systematic Explorer (EASE)

- Theme discovery: identification of Gene Ontology (GO) terms that describe a statistically significant number of genes in the list compared to the population of genes from which the list was derived
- Employs a variation of the one-tailed Fisher exact probability for over-representation ("EASE score")
- To address multiple comparison problem (significant probabilities arising simply due to chance when calculating statistics on thousands of genes), EASE employs several probability corrections, including Bonferroni, false discovery rate, and bootstrap methods

Functional Categories Over-Represented in Cyst and Tumor vs. Surface Epithelium

- Down-regulated: signal transduction activity \((n = 31); P = 0.004\)
- Up-regulated: mitotic cell cycle \((n = 12); P = 0.03\)
- Up-regulated: microtubule organization and biogenesis \((n = 6); P = 0.007\)
Hypothesis

- Attenuated transduction of extracellular (or intracellular) signals leads to inappropriate cell cycle progression that when coupled with defective mitotic spindle assembly, promotes the development of aneuploidy
- p53 overexpression in normal (and dysplastic) cystic epithelium reflects oncogenic stress in the form of activated oncogenes, DNA damage resulting from inappropriate cell cycle progression, and defective mitotic spindle assembly

Increased mitotic activity in cystic epithelium compared to surface epithelium?

Assessment of Cell Proliferation by Ki-67 IHC

<table>
<thead>
<tr>
<th>Cell type</th>
<th># samples</th>
<th># nuclei</th>
<th># positive nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovaries</td>
<td>36</td>
<td>39,832</td>
<td>220 (0.55%)</td>
</tr>
<tr>
<td>Surface</td>
<td>28</td>
<td>19,216</td>
<td>64 (0.33%)</td>
</tr>
<tr>
<td>Cystic</td>
<td>32*</td>
<td>20,616</td>
<td>156 (0.76%)</td>
</tr>
</tbody>
</table>

*159 inclusion cysts  \( P = 0.0001 \)

Rate of apoptosis in cyst vs. surface?

(TUNEL assay)
### Apoptosis

<table>
<thead>
<tr>
<th>Cell type</th>
<th># samples</th>
<th># nuclei</th>
<th># positive nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovaries</td>
<td>12</td>
<td>10,270</td>
<td>398 (3.9%)</td>
</tr>
<tr>
<td>Surface</td>
<td>9</td>
<td>2,197</td>
<td>274 (12%)</td>
</tr>
<tr>
<td>Cystic</td>
<td>10*</td>
<td>8,073</td>
<td>124 (1.5%)</td>
</tr>
</tbody>
</table>

*89 inclusion cysts

\[ P = 0.0001 \]

### “Cell Proliferation Index”

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Ki-67</th>
<th>Apoptosis</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>0.33%</td>
<td>12%</td>
<td>0.028</td>
</tr>
<tr>
<td>Cystic</td>
<td>0.76%</td>
<td>1.5%</td>
<td>0.51</td>
</tr>
<tr>
<td>Cyst to surface</td>
<td>18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### DNA Content in Archival Ovarian Tissues

- FFPE normal ovaries cut into 10 μM sections (to avoid sectioning through nuclei
- Aneuploid MCF7 and SKBR3 breast cancer cell lines used as positive controls
- Slides deparaffinized and stained with propidium iodide
- Slides imaged using Zeiss Axioplan 2 microscope

### DNA Content in Archival Ovarian Tissues

- Images captured at 400X with AxiosCam MR monochrome digital camera providing a resolution of 1300 x 1030 pixels using the ApoTome (enhanced) confocal imaging system
- Using AxiosVision image acquisition software, 7 optical sections are captured per high-power field at 0.85 μM intervals
- Monochrome images analyzed using the MetaMorph® Imaging System
- Nuclei outlined manually with region tracing tools
  - Integrated intensity over the total area of the identified nucleus is calculated
  - Each pixel is given an intensity value from 0 (pure black) to 255 (pure white)
  - Overlapping nuclei are excluded from the analysis

### ApoTome

- ApoTome image (left) compared to conventional wide-field confocal image (right). ApoTome image is sharper and better focused.
Nuclear Image Acquisition and Quantitation

Propidium iodide stained ovarian epithelium

Monochrome image acquired

Non-overlapping nuclei outlined manually; region intensity values measured in seven optical sections; greatest value is included in analysis

DNA Content

<table>
<thead>
<tr>
<th>Cell type</th>
<th># nuclei</th>
<th>Mean intensity/nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>78</td>
<td>127,215</td>
</tr>
<tr>
<td>Cystic</td>
<td>217</td>
<td>151,831</td>
</tr>
<tr>
<td>Aneuploid control</td>
<td>82</td>
<td>309,877</td>
</tr>
<tr>
<td>Total</td>
<td>377</td>
<td>181,114</td>
</tr>
</tbody>
</table>

P = 0.001

Karyotypic aneuploidy?

(FISH using centromeric probes for chromosomes 1,3,6,7,8,11)

MCF7 cells (aneuploid)
Surface epithelium

Inclusion cyst

Inclusion cyst

Inclusion cyst

Inclusion cyst

Aneuploidy

<table>
<thead>
<tr>
<th>Ploidy</th>
<th>Surface</th>
<th>Cystic</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Total cells)</td>
<td>410</td>
<td>880</td>
</tr>
<tr>
<td>Diploid</td>
<td>408 (99.5%)</td>
<td>799 (90.8%)</td>
</tr>
<tr>
<td>Aneuploid</td>
<td>2 (0.5%)</td>
<td>81 (9.2%)</td>
</tr>
</tbody>
</table>

\[ P = 0.001 \]
Conclusions

- A substantial proportion of ovarian carcinomas arise in cystic inclusions of the surface epithelium
- A limited field of dysplasia arises from normal-appearing cyst epithelium, which gives rise to invasive carcinoma
- The molecular phenotype of pathologically normal cystic epithelium is quasi-neoplastic
  - Molecular profile suggests oncogenic stress, altered signal transduction, increased mitosis, defective mitotic spindle assembly
  - Experimental evidence indicates increased mitotic index and aneuploidy in pathologically normal ovarian epithelial cystic inclusions
  - Aneuploidy is a very early, possibly critical event in ovarian tumorigenesis

Implications

- Elucidation of the very early molecular alterations relevant to ovarian tumorigenesis could be accomplished through the analysis of normal epithelium and dysplastic lesions within epithelial inclusion cysts
- Novel strategies for the early diagnosis of ovarian carcinoma may reasonably be directed toward the detection of this histopathologic phenomenon (e.g., through molecular imaging)

Acknowledgments

GBRL
Bhavana Pothuri, M.D.
Mario Leitao, M.D.
Doug Levine, M.D.
Genomics Core Lab
Agnès Viale, Ph.D.
Dept Epidemiol/Biostat
Adam Olshen, Ph.D.

Dept Pathology
Robert Soslows, M.D.
Oscar Lin, M.D.

Dept Medicine
Kenneth Offit, M.D.
Mark Robson, M.D.

Dept Surgery
Richard Barakat, M.D.

NIH
R01 CA71840
Screening for Ovarian Cancer in High Risk Women

Patricia Hartge, Sc.D.
Deputy Director
Epidemiology and Biostatistics Program
Division of Cancer Epidemiology and Genetics
National Cancer Institute

Overview of Ovarian Cancer Risk
- Age-specific incidence
- International variation
- US geographic pattern
- Lifetime risks of developing
- Risk factors and protective factors

Age-Specific Incidence Rates

Incidence Varies Between Countries

Summary Lifetime Risks

<table>
<thead>
<tr>
<th></th>
<th>US White</th>
<th>US Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability of developing</td>
<td>1.4 %</td>
<td>0.8 %</td>
</tr>
<tr>
<td>Birth to 39</td>
<td>0.07 %</td>
<td>0.06 %</td>
</tr>
<tr>
<td>40-59</td>
<td>0.4 %</td>
<td>0.2 %</td>
</tr>
<tr>
<td>60-84</td>
<td>1.1 %</td>
<td>0.7 %</td>
</tr>
<tr>
<td>Probability of dying from</td>
<td>1.0 %</td>
<td>0.6 %</td>
</tr>
</tbody>
</table>
Risk and Protective Factors

Performance of TVU and CA-125

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity %</th>
<th>95% CI</th>
<th>PPV %</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVU</td>
<td>46.2</td>
<td>19.1 - 73.3</td>
<td>17.1</td>
<td>4.7 - 29.6</td>
</tr>
<tr>
<td>Serum CA-125</td>
<td>81.8</td>
<td>59.0 - 100</td>
<td>63.4</td>
<td>39.2 - 89.4</td>
</tr>
</tbody>
</table>

Abbreviations: TVU, transvaginal ultrasound; PPV, positive predictive value.

Sterling et al. 2005

CA-125 Outcome Measurements

Sterling et al. 2005

Screening for Familial Ovarian Cancer: Failure of Current Protocols to Detect Ovarian Cancer at an Early Stage According to the International Federation of Gynecology and Obstetrics


Screening for Familial Ovarian Cancer: The Need for Well-Designed Prospective Studies

Ovarian Cancer Screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial: Findings from the Initial Screen of a Randomized Trial


Characteristics of participants in the intervention arm

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE GROUP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55 - 59</td>
<td>1349</td>
<td>34.4</td>
</tr>
<tr>
<td>60 - 64</td>
<td>11774</td>
<td>30.1</td>
</tr>
<tr>
<td>65 - 69</td>
<td>8588</td>
<td>22.0</td>
</tr>
<tr>
<td>70 - 74</td>
<td>5294</td>
<td>13.5</td>
</tr>
<tr>
<td>RACE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>33828</td>
<td>86.5</td>
</tr>
<tr>
<td>Black</td>
<td>2170</td>
<td>5.5</td>
</tr>
<tr>
<td>Hispanic</td>
<td>605</td>
<td>1.5</td>
</tr>
<tr>
<td>Asian</td>
<td>1259</td>
<td>3.2</td>
</tr>
<tr>
<td>Other</td>
<td>283</td>
<td>0.7</td>
</tr>
<tr>
<td>Missing Response</td>
<td>970</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Characteristics of participants in the intervention arm (continued)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDUCATION LEVEL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; High school</td>
<td>2920</td>
<td>6.4</td>
</tr>
<tr>
<td>12yrs/Completed High School</td>
<td>15299</td>
<td>39.1</td>
</tr>
<tr>
<td>Some College</td>
<td>8911</td>
<td>22.8</td>
</tr>
<tr>
<td>College Graduate</td>
<td>5828</td>
<td>14.9</td>
</tr>
<tr>
<td>Postgraduate</td>
<td>5557</td>
<td>14.2</td>
</tr>
<tr>
<td>Missing Response</td>
<td>1020</td>
<td>2.6</td>
</tr>
<tr>
<td>HAD PRIOR PELVIC SURGERY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral Oophorectomy</td>
<td>64</td>
<td>0.2</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>9065</td>
<td>23.2</td>
</tr>
<tr>
<td>Bilateral Oophorectomy &amp; Hysterectomy</td>
<td>4789</td>
<td>12.2</td>
</tr>
<tr>
<td>Neither</td>
<td>24114</td>
<td>61.6</td>
</tr>
<tr>
<td>Missing Response</td>
<td>1083</td>
<td>2.8</td>
</tr>
<tr>
<td>EVER TAKEN ORAL CONTRACEPTIVES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>17622</td>
<td>45.1</td>
</tr>
<tr>
<td>Yes</td>
<td>20465</td>
<td>52.3</td>
</tr>
<tr>
<td>Missing Response</td>
<td>1028</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Flow of participants into the PLCO Trial

Follow-up of positive screens

Follow-up of positive screens (con’t)
Preliminary Data – PLCO Positive Screens Separated by Familial Risk

Breast cancer rates and relative risks according to recognized breast cancer risk factors in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial Cohort

Lacey JV, Buys SS, Marcus P, Chang S-C, Leitzmann MF, Hoover RN, Prorok PC, Berg CD, and Hartge P, for the PLCO Project Team. (Submitted)

PLCO Breast Cancer Risk

Ongoing Research

- Completion of the trial
- Only accurate assessment of efficacy
- Preliminary findings on high risk women
- Examination of additional markers
New Directions in Ovarian Cancer Epidemiology
Joellen M. Schildkraut, Ph.D.
Duke University Medical Center

Occurrence of Ovarian Cancer
• 25,200 new cases / yr in the U.S.
• Often asymptomatic until it reaches an advanced stage.
• Survival is poor with 14,500 deaths / yr.
• Practical screening approaches are not available at this time.

Histologic Features

<table>
<thead>
<tr>
<th>Overall Frequency</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germ Cell</td>
<td>15%-20%</td>
</tr>
<tr>
<td>Teratoma</td>
<td>5%-8%</td>
</tr>
<tr>
<td>Dysgerminoma</td>
<td></td>
</tr>
<tr>
<td>Endodermal sinus</td>
<td></td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex cord-stromal</th>
<th>5%-10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroma</td>
<td>2%-3%</td>
</tr>
<tr>
<td>Granulosa-theca cell</td>
<td></td>
</tr>
<tr>
<td>Serotoli-Leydig cell</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Epithelial tumors</th>
<th>65%-70%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>90%</td>
</tr>
<tr>
<td>Mucinous</td>
<td></td>
</tr>
<tr>
<td>Endometioid</td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td></td>
</tr>
<tr>
<td>Brenner</td>
<td></td>
</tr>
<tr>
<td>Cystadenofibroma</td>
<td></td>
</tr>
</tbody>
</table>

Epidemiology: Risk Factors

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>2.1</td>
</tr>
<tr>
<td>Oral Contraceptive Use</td>
<td>0.30</td>
</tr>
<tr>
<td>Infertility</td>
<td>2.1</td>
</tr>
<tr>
<td>Tubal Ligation</td>
<td>0.87</td>
</tr>
<tr>
<td>Family History of Ovarian Cancer in a 1o relative</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Genetic Susceptibility
• BRCA1 and BRCA2 mutations carriers have high lifetime risk for developing ovarian cancer:
  - BRCA1 ~ 40%
  - BRCA2 ~ 25%
• Risk may be reduced by*:
  - OC use: OR = 0.44 (BRCA1)
  - OR = 0.35 (BRCA2)

* Narod et al. Lancet 2001

Genetic Susceptibility
• BRCA1 and BRCA2 mutations account for ~10% of ovarian cancers

Mismatch repair genes (hMSH2, hMLH1, hPMS1, and hPMS2) observed in nonpolyposis colorectal syndrome (HNPCC) account for ~1-2% of ovarian cancer
• Lifetime risk for ovarian cancer by age 50 in HNPCC families with mutation in MSH2 is ~20%.
Pathogenic Models for Ovarian Cancer

The Incessant Ovulation Hypothesis:
- Proliferation induced mutations
- Increased gonadotropins
- Increased inclusion cyst formation

Factors that decrease lifetime ovulatory cycles decrease risk of ovarian cancer:
- OC use
- Pregnancy
- Breastfeeding

Pathogenic Models (continued)

Progestin-Apoptosis Theory: Progestin induced apoptosis may protect against the development of ovarian cancer
- Pregnancy and OC use are associated with increased exposure to progestin.
- Ovarian epithelium contains receptors for estrogen, progesterone, and androgen.
- Reproductive factors may affect ovarian cancer via biologic interaction between sex steroids and the ovarian epithelium.

Pathogenic Models (continued)

Inflammation Theory:
Inflammatory reaction induced by ovulation, asbestos and talc exposure, endometriosis, and pelvic inflammatory disease leads to DNA damage in the inflammation-induced inclusion cysts.

Study Team
Joellen Schildkraut, Ph.D.
Andrew Berchuck, M.D.
Patricia Moorman, Ph.D.
Ed Iversen, Ph.D.
Jeff Marks, Ph.D.
Susan Halabi, Ph.D.
Susan Murphy, Ph.D.
Brian Calingaert, MS, MBMA
Christine Lankevich, MPH
Regina Whitaker, BS
Toya Hobb, RN
Adrian Johnson, RN
Whitney Franz, RN

PURPOSE
- To understand the mechanism(s) of ovarian cancer development
- To define disease subsets on the basis of molecular signatures and other patient and tumor characteristics
- To be able to target high risk population for prevention and screening strategies
Hypothesis

Since a greater number of ovulatory cycles increases the risk of ovarian cancer by inducing proliferation-associated DNA damage we hypothesized that ovulation is associated with p53-positive ovarian cancer but not p53-negative tumors.

Methods

Study Subjects: 197 women with ovarian cancer and 3363 control subjects who participated in the Cancer and Steroid Hormone (CASH) study, aged 20-54.

Case-control comparison of the number of lifetime ovulatory cycles by p53 status: p53-positive vs. control subjects

<table>
<thead>
<tr>
<th>No. of ovulatory cycles</th>
<th>No. of p53-positive cases</th>
<th>No. of control subjects</th>
<th>OR*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 234</td>
<td>4</td>
<td>840</td>
<td>1.0</td>
<td>Referent</td>
</tr>
<tr>
<td>235-375</td>
<td>29</td>
<td>1222</td>
<td>4.3</td>
<td>1.4-13.0</td>
</tr>
<tr>
<td>376-533</td>
<td>67</td>
<td>1159</td>
<td>9.1</td>
<td>2.7-30.9</td>
</tr>
</tbody>
</table>

*Adjusted by age, age², menopausal status, and nulliparity.

Case-control comparison of lifetime ovulatory cycles: p53-negative vs. control subjects

<table>
<thead>
<tr>
<th>No. of ovulatory cycles</th>
<th>No. of p53-negative cases</th>
<th>No. of control subjects</th>
<th>OR*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 234</td>
<td>23</td>
<td>840</td>
<td>1.0</td>
<td>Referent</td>
</tr>
<tr>
<td>235-375</td>
<td>19</td>
<td>1222</td>
<td>0.6</td>
<td>0.3-1.4</td>
</tr>
<tr>
<td>376-533</td>
<td>45</td>
<td>1159</td>
<td>1.3</td>
<td>0.5-3.2</td>
</tr>
</tbody>
</table>

*Adjusted by age, age², menopausal status, and nulliparity.

STUDY DESIGN

- Population-Based, Case-Control Study
- Newly Diagnosed/Incident Epithelial Ovarian Cancer (borderline & invasive)
- RDD identification of Controls
- 48-County Region in North Carolina

North Carolina Ovarian Cancer Study
**DATA COLLECTION**
- 9-year Period (starting 1/1/99)
- Rapid-case ascertainment
- Physician Approval
- Invitation Letter (toll free number)
- Financial Incentive
- Written Informed Consent
- In-person Interview (nurse interviewer)
- Blood sample & tumor tissue

**ENROLLMENT**
(as of 8/2005)

<table>
<thead>
<tr>
<th>SUBJECT TYPE ENROLLED</th>
<th>COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial Ovarian Cancer Patients</td>
<td>786</td>
</tr>
<tr>
<td>Peritoneal Cancer Patients</td>
<td>62</td>
</tr>
<tr>
<td>Control Subjects</td>
<td>831</td>
</tr>
<tr>
<td>Total</td>
<td>1679</td>
</tr>
</tbody>
</table>

**Objective**
To examine the relationship between overexpression of the cyclin E oncogene and ovarian cancer risk. Cyclin appears to be involved in cell proliferation and may play a role in aneuploidy.

**Case-control comparison of lifetime ovulatory cycles:**

<table>
<thead>
<tr>
<th>Cyclin E-positive vs. control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ovulatory cycles</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>&lt; 265</td>
</tr>
<tr>
<td>265-390</td>
</tr>
<tr>
<td>&gt; 390</td>
</tr>
</tbody>
</table>

*Adjusted by age, age², race, menopausal status

**Case-control comparison of Oral Contraceptive Use (OC):**

<table>
<thead>
<tr>
<th>Cyclin E-positive vs. control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years of OC Use</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>≤ 5</td>
</tr>
<tr>
<td>&gt; 5</td>
</tr>
</tbody>
</table>

*Adjusted by age, age², race, menopausal status
Case-control comparison of Oral Contraceptive Use (OC): Cyclin E-negative vs. control subjects

<table>
<thead>
<tr>
<th>Years of OC Use</th>
<th>No. of Cyclin E negative cases</th>
<th>No. of control subject</th>
<th>OR*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55</td>
<td>219</td>
<td>1.0</td>
<td>Referent</td>
</tr>
<tr>
<td>≤ 5</td>
<td>65</td>
<td>283</td>
<td>0.9</td>
<td>0.6-1.3</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>52</td>
<td>215</td>
<td>0.9</td>
<td>0.6-1.5</td>
</tr>
</tbody>
</table>

*Adjusted by age, age², race, menopausal status

Objective

To examine the relationship between analgesic use and the risk of epithelial ovarian cancer using case-control data from the North Carolina Ovarian Cancer Study (NCOCS).

Conclusions

- These data support an inverse relationship between the use of both NSAIDS and acetaminophen and the risk of ovarian cancer.
- The high prevalence of analgesic use has implications for a significant reduction in the number of ovarian cancer cases diagnosed each year.

New Directions

Objective

To determine whether the progesterone receptor promoter +331A polymorphism affects the risk of ovarian cancer.
Odds ratios and 95% CIs for association between the PR Promoter +331A and epithelial ovarian tumors by histologic subtype

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Cases</th>
<th>AG</th>
<th>AA</th>
<th>AG/AA (%)</th>
<th>OR*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>445</td>
<td>58</td>
<td>1</td>
<td>59 (11.7)</td>
<td>1.00</td>
<td>Reference</td>
</tr>
<tr>
<td>Serous</td>
<td>244</td>
<td>26</td>
<td>0</td>
<td>26 (9.6)</td>
<td>0.81</td>
<td>(0.50 - 1.32)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>44</td>
<td>5</td>
<td>0</td>
<td>5 (10.2)</td>
<td>0.80</td>
<td>(0.30 - 2.14)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>53</td>
<td>3</td>
<td>0</td>
<td>3 (5.4)</td>
<td>0.43</td>
<td>(0.13 - 1.40)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0 (0.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Endometrioid/clear cell</td>
<td>76</td>
<td>3</td>
<td>0</td>
<td>3 (3.8)</td>
<td>0.30</td>
<td>(0.09 - 0.97)</td>
</tr>
<tr>
<td>Mixed</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0 (0.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>35</td>
<td>3</td>
<td>1</td>
<td>4 (10.3)</td>
<td>0.86</td>
<td>(0.29 - 2.49)</td>
</tr>
</tbody>
</table>

*ORs are for genotype (AG/AA) compared to reference genotype GG and are adjusted for age.

**Conclusion**

The +331G/A progesterone receptor polymorphism may modify the molecular epidemiology pathway that encompasses the development of endometrioid/clear cell ovarian cancer possibly via subsequent transformation from endometriosis.

**New Directions**

**SUMMARY**

Epidemiologic data suggest that prevention strategies that target the active management of ovulation, such as oral contraceptives, may be effective for reducing the risk of ovarian cancer. Not all ovarian cancers may be due to high ovulation exposure and therefore other chemopreventative agents may be considered.

Identification of subsets of women at risk for ovarian cancer will facilitate chemopreventive interventions. Refining our ability to define a population at risk for ovarian cancer will increase the efficacy of both prevention and screening strategies.
GENERAL MODEL FOR MOLECULAR EPIDEMIOLOGICAL STUDIES

Epidemiological framework
Meta analysis, case-control, cohort...

Laboratory studies of biomarkers:
Exposure, susceptibility, Early detection...

Mechanistic outcome — Epidemiological Outcome
Incidence, Relative Risk, Odds Ratio

FINAL PURPOSE

To integrate mechanistic and epidemiological outcomes to understand disease etiology and develop public health interventions

Why add biomarkers?

ASSOCIATIONS THAT TAKE INTO ACCOUNT MORE COMPLEX BIOCHEMICAL AND MOLECULAR MECHANISMS SHOULD BE STRONGER

REQUIREMENTS

NEW CONCEPTUAL AND METHODOLOGICAL APPROACHES ARE NEEDED FOR SUCH STUDIES
Pregnancy, Hormones & Ovarian Cancer

Paolo Toniolo, MD
Annekatrin Lukanova, MD, PhD
Division of Epidemiology
Dept. of Obstetrics & Gynecology
New York University School of Medicine

Pregnancy and Ovarian Cancer

- parity: consistent protection across studies
- ↓ risk: 30-70%
  10-15% per birth

Ovulation cessation of pregnancy

greater protection than:

- 12- months OC use
- delayed menarche
- early menopause

Protection from Pregnancy

- 1st FTP
- twin pregnancies
- late age at 1st FTP
- late age at last FTP

Why Pregnancy Protects?

Traditional hypotheses
- Incessant ovulation
- Excess gonadotropin

More recent thinking
- Washout effect

Washout Effect Hypothesis

(Adami et al, 1994)

- Epithelial layers cleared of transformed cells
- Likelihood that transformed cells are present increases with age
- Benefit from the elimination of initiated cells diminishes with time since pregnancy
Washout Effect

- Pregnancy hormones
- Progesterone
  - Pro-apoptotic & growth inhibition of OSE and ovarian cancer cells
  - Expression of PR associated with favorable prognosis

Progesterone in Pregnancy and Risk of Maternal Ovarian Cancer

A prospective epidemiological study in Nordic countries

Two Large Cohorts

- North Sweden Maternity Cohort
  - Established 1985
  - > 110,000 1st trimester samples (85,000 women) stored at -20C

- Finnish Maternity Cohort
  - Established 1993, nationwide
  - > 1.3 million 1st trimester samples (850,000 women), stored at -25C

Study Aims

- Maternal risk of epithelial ovarian cancer associated with circulating
  - Progesterone (P)
  - 17α-OH progesterone (OHP)
  during the 1st trimester of a last FTP
- Effect modification of age at last FTP
- Effect modification of histological subtypes

Study Design

- Case-control study nested within the two cohorts
- Cases: All new epithelial cases, 1983-2008
  - Invasive + borderline
- Controls: Cohort members (2:1), matched on
  - Cohort
  - Age
  - Date at blood draw
  - Parity at blood draw
**Nested Case-Control Sampling**

- Case
  - Cancer
- Controls
- Time
  - Present

**Study Design**

- **Eligibility:**
  - Last pregnancy of one infant at term (37+ weeks)
  - Blood sampling between 7th and 14th week
- **Case identification:** nationwide tumor registries
- **Data:** linkages, Population & Birth Registries
  - Pregnancy order, twin pregnancies, induced pregnancies, hormonal treatments, non-term

**Expected Number of Cases**

<table>
<thead>
<tr>
<th>Site</th>
<th>Borderline</th>
<th>Invasive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finland</td>
<td>687</td>
<td>957</td>
<td>1644</td>
</tr>
<tr>
<td>Sweden</td>
<td>56</td>
<td>127</td>
<td>183</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>743</strong></td>
<td><strong>1084</strong></td>
<td><strong>1827</strong></td>
</tr>
</tbody>
</table>

**Contributors**

- **NYU School of Medicine**
  - Yelena Afanasyeva
  - Alan Arslan
  - Annekatrin Lukanova
  - Paolo Toniolo
  - Anne Zeleniuch-Jacquotte

- **Finnish National Public Health Institute**
  - Matti Lehtinen
  - Eero Pukkala
  - Elisabete Weiderpass

- **University of Umeå**
  - Kjell Grankvist
  - Goran Hallmans
  - Eva Lundin
  - Goran Wadell
  - Marianne Wulff
Modeling Epithelial Ovarian Cancer in the Mouse

Denise C. Connolly, Ph.D.
Ovarian Cancer Program
Fox Chase Cancer Center, Philadelphia, PA

Ovarian cancer
- Fifth most common cancer among women in the United States
- ~24,000 new cases diagnosed annually
- ~15,000 deaths annually
- Survival rate ~ 90% (early diagnosis)
- Majority diagnosed at late stage
  - 5 year survival rate is 30-40%

Ovarian cancer risk
- Nulliparity ↑
- Fertility drug use ?
- Multiparity ↓
- Oral contraceptive use ↓
- Family history – accounts for 5-10% of cases

Histogenesis of ovarian neoplasms
- Epithelium – (85-90%)
  - ovarian carcinomas: serous, endometrioid, mucinous, clear cell
- Follicles, cortical stroma, hilum
  - Sex cord stromal tumors:
    - granulosa, Sertoli, Leydig, theca
- Oocytes
  - Germ cell tumors:
    - dysgerminoma, teratoma, embryonal CA

Genetic changes in ovarian cancer
- Oncogenes
  - Myc
  - K-Ras
  - ErbB2
  - EGF-R
  - PI3K
  - Akt
  - STAT3
- Tumor Suppressors
  - p53
  - BRCA1 and BRCA2
  - PTEN
- Genomic instability
- DNA modification (promoter methylation)

Animal models of epithelial ovarian cancer (EOC)
- Spontaneous- aging hens
- Chemical carcinogen induced- DMBA rodent models
- Xenograft models- transplanted human tumor cell lines
- Syngeneic models of in vitro transformed rodent ovarian surface epithelium (OSE)
- Genetically engineered mouse (GEM) models
Why model cancer in mice?

- Establish causal events
  - Gene mutations, cellular alterations
- Identify early lesions and events occurring at each stage
  - Order events, compare relative importance
- Flexibility in interbreeding
  - Test cooperative effects of genetic alterations
- Identify other genes that impact tumorigenesis
  - Genetic modifiers that affect disease penetrance
- Evaluate therapeutic, detection and prevention strategies

Timeline of GEM model technology

- Early-mid 1980’s: Transgenic mice
- Late 1980’s: Homologous recombination
  - Knockout
  - Knock-in
- Mid-late 90’s: TVA transgenics
- Late 1990’s: Cre-LoxP mediated conditional
  - Inactivation
  - Activation
- 2001-Present: Recombineering/BAC’s

Why the delay?

- Lack of understanding of epithelial precursor
- 75% of patients diagnosed at an advanced stage when tumors contain numerous genetic alterations that are too complex for biochemical characterization
- Incomplete understanding of tumor initiating pathways
- Difficult to identify pathways that are necessary for tumor maintenance

Transgenic mice

- Introduction of genes into the germline of fertilized eggs
- First examples described mid 1980’s
- Requires a tissue specific or restricted gene promoter to target transgene expression to the organ/tissue of interest

Candidate validation

1) RT-PCR
   - normal mouse organs, primary cultures of MOSE cells, enriched (uncultured) populations of MOSE cells and transformed MOSE cells
2) Obtain promoter and confirm transcriptional activation in cell culture
   - Reporter gene assays
3) Transgenic mice
   - SV40 TAg – Functional inactivation of p53 and RB

Promoter identification strategies

- Theoretical: (e.g., FSHR and MIIIR)
- Literature review: (e.g., Mesothelin)
- Experimental: (e.g., CK19)
Müllerian inhibitory substance (MIS) signaling

Males – MIS is secreted by the Sertoli cells in developing testes. This hormone sequentially binds to the MIS type II and type I receptors, and signals regression of the Müllerian duct.

Females – In the absence of MIS and its subsequent signaling via the type II and type I receptors, the Müllerian duct persists to give rise to the epithelia of the uterus, Fallopian tube and cervix.

RT-PCR analysis of MISIIR expression in mouse tissues

Mouse MISIIR promoter

- PCR cloned based on homology to published rat MISIIR promoter sequence and mouse genomic DNA sequences
  - 1204 bp fragment that shares >95% sequence homology to rat promoter

In Vitro transcriptional activity of the mouse MISIIR promoter

MISIIR-TAg construct

- Murine 5’ upstream regulatory region of the Mullerian inhibiting substance type II receptor (MISIIR) gene PCR fused to the early region of SV40 including the large and small T antigen genes (provided by D. Hanahan)
Bilateral ovarian tumors in a MISIR-TAg transgenic mouse

ScanScope view of ovaries substituted by tumor cells

Tubular and papillary structures and floating buds in intrabursal space

Papillary structures apparent in ascites and invasion of omentum

Histological Features
- Poorly differentiated carcinomas with cysts and papillary structures present at the surface of the ovary
- Intraperitoneal dissemination with invasion of omentum, implants in peritoneal organs and formation of ascites

Tumors express TAg and p53
Tumors express markers of epithelial cell differentiation

Immunohistochemical Features
- Majority of tumor cells contained nuclear TAg both in ovarian tumors and intraperitoneal masses
- Elevated p53 protein in tumor cells
- Cytokeratins 8 and 19 are uniformly expressed in the majority of tumor cells
- α-Inhibin is expressed in granulosa cells of remaining normal follicles, but not in tumor cells

Mouse Ovarian Carcinoma (MOVCAR) cell lines
- Derived from the ascites of mice with tumors
- Exhibit anchorage independent growth in soft agar
- Tumorigenic in immunocompromised mice (SCID)
  - Tumors exhibit similar histological features, expression of epithelial cell markers, absence of α-inhibin
  - Organotropic implantation

Stable transgenic lines?
- Most female mice develop ovarian tumors prior to breeding
- ~28% of males develop Sertoli cell tumors
- Some female offspring of affected males develop ovarian tumors
- Targeting reproductive organs for tumorigenesis = poor breeding

DR26 transgenic mice
- Status:
  - Backcrossed to C57Bl/6
  - Males fertile
  - Females tested infertile
  - Female mice develop bilateral ovarian tumors
  - Average lifespan = 130 days
  - Most develop ascites

Early Lesions?

Connolly
Four week-old mice:

Therapeutics

- TgMISIIR-TAg mice have variable disease latency
- How to design therapeutic experiments?
  - Experimental endpoint with age matched controls?
  - Survival as endpoint?
  - Can each animal serve as its own control?
- *In vivo* imaging

Magnetic Resonance Imaging (MRI) of mice

- Images acquired with a 2-D spin-echo pulse sequence, TR=1200 msec, TE=13 msec
- Dedicated 7 Tesla animal scanner
- 4 averages, total imaging time 19 min.
- Contrast – i.m. injection Gd-DTPA
- Tumor volumes calculated using MRicro
  [http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html](http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html)
Volume Measurement

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>256 pixels</td>
</tr>
<tr>
<td>Y</td>
<td>256 pixels</td>
</tr>
<tr>
<td>Z</td>
<td>32 slices</td>
</tr>
</tbody>
</table>

Formula: \((0.1 \text{ mm})(0.1 \text{ mm})(0.5 \text{ mm})(n\text{ROI})\)
Cisplatin/Taxol treatment

Similarities between TgMISIIR-TAg mouse and human EOC

- Disease presentation:
  - Asymptomatic
  - Advanced stage
  - Peritoneal spread, ascites
- Histology:
  - Serous carcinoma
- Markers:
  - Cytokeratin 8 and 19 expression
  - Muc16 (murine CA125) – J. Boyd
  - P-AKT2 expression – J. Testa
  - Common genes/pathways altered in mouse and human ovarian cancers (identified by cDNA arrays)

Future Goals

- Test efficacy of therapeutic agents in TgMISIIR-TAg mice using MRI to measure tumor burden
- Study the impact of fertility and reproduction on the development of EOC
- Develop genetically relevant mouse models of human EOC
  - MISIIR promoter to express oncogenes in transgenic mice
  - Conditional Cre-LoxP mediated inactivation of tumor suppressor genes

Acknowledgments
MECHANISM OF OVARIAN CANCER Predisposition IN INDIVIDUALS WITH GERMLINE BRCA1 MUTATIONS

Louis Dubeau, M. D., Ph. D.
USC/Norris Comprehensive Cancer Center
Keck school of Medicine of University of Southern California

SELECTIVE DISADVANTAGE OF REDUCED BRCA1 EXPRESSION

INFLUENCE OF PARITY AND ORAL CONTRACEPTIVE USE ON OVARIAN CANCER RISK

Risk Factors | Relative Risk
---|---
Parity 0 | 1.00
1 | 0.61
2 | 0.43
3 | 0.40
4+ | 0.31
Total Oral Contraceptive | 0.9
<1 | 0.8
1-3 | 0.5
3-5 | 0.4
5+ | 0.4

SECONDARY FOLLICLE

Oocyte
Granulosa cells
MECHANISM OF OVARIAN CANCER PREDISPOSITION IN BRCA1 MUTATION CARRIERS

Cell non-autonomous hypothesis:

Cell of origin → Ovarian carcinoma

MUTANT MICE DEVELOP OVARIAN CYSTADENOMAS

INVASIVE CARCINOMA IN A P53/BRCA1 DOUBLE MUTANT

IMMUNOHISTOCHEMICAL STAINING FOR MULLERIAN INHIBITING SUBSTANCE

IMMUNOHISTOCHEMICAL STAINING FOR NON-SQUAMOUS KERATINS
TISSUE DISTRIBUTION OF MUTANT ALLELES

**BRCA1 exon 11**

- rearranged allele (a-c primers)
- unrearranged allele (a-b primers)

**MW markers**

- Tail
- Ovary
- Tumor epithelium
- Cyst wall
- Uterine horn
- No DNA

**MECHANISM OF OVARIAN CANCER PREDISPOSITION IN BRCA1 MUTATION CARRIERS**

- Reduced BRCA1 expression

**Cell of origin**

- Ovarian neoplasia

**PERI-TUBAL CYST IN BRCA1 KNOCK-OUT MICE**

- DC mutant mice develop epithelial cysts along the entire Mullerian tract

**UTERINE CYST**

**COMMENTARY**

The Cell of Origin of Ovarian Epithelial Tumors and the Ovarian Surface Epithelium Dogma: Does the Emperor Have No Clothes?

Louis Dubeau

USC Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, California, USA
POSSIBLE MECHANISMS FOR OVARIAN TUMOR PREDISPOSITION BASED ON A CELL NON-AUTONOMOUS SCENARIO

- Direct mechanism
  - An effector secreted by granulosa cells is regulated by Brca1

- Indirect mechanism
  - Brca1 inactivation results in alterations in the ovulatory cycle, which in turn influences ovarian epithelial tumor development

DIFFERENCES IN PCNA EXPRESSION IN ENDOMETRIAL STROMA OF WILD TYPE VERSUS MUTANT MICE

<table>
<thead>
<tr>
<th>Age: 3-4 months</th>
<th>Estrus cycle stage: early diestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>36.1 +/- 24.8%</td>
</tr>
<tr>
<td>Mutant</td>
<td>67.7 +/- 6.7%</td>
</tr>
<tr>
<td>P = .025</td>
<td></td>
</tr>
</tbody>
</table>

Classification of estrus cycle stages in vaginal smears using the Papanicolaou stain

Diffusions in Proliferation Cell Nuclear Antigen (PCNA) expression in endometrial stroma of wild type versus mutant mice. The images show differences in PCNA expression levels, with mutant mice having significantly higher expression levels compared to wild type mice. The classification of estrus cycle stages includes:

- Diestrus
- Proestrus
- Preestrus/Estrus

Classification of estrus cycle stages in vaginal smears using the Papanicolaou stain

Modulation of Aromatase Expression by BRCA1: a Possible Link to Tissue-Specific Tumor Suppression

Yanfen Hu, Sagar Ghosh, Asma Amleh, Wei Yue, Yanzhe Lu, Adam Katz, Rong Li

Department of Biochemistry and Molecular Genetics
Department of Medicine and Division of Endocrinology
School of Medicine
University of Virginia
Charlottesville, VA 22908

State Key Laboratory of Genetic Engineering
Institute of Genetics
School of Life Science
Fudan University
Shanghai 200433, China

Oncogene, in press
COMPARISON OF LENGTH OF ESTRUS CYCLE STAGES IN MUTANT VERSUS WILD-TYPE LITTERMATE MICE

SUMMARY

- The embryonic lethality of BRCA1 general knock out was avoided by knocking out this gene in granulosa cells specifically.
- Mouse carrying mutant alleles of BRCA1 in their granulosa cells had morphologically normal ovarian follicles and were fertile, but developed benign and malignant epithelial ovarian tumors as well as extra-ovarian cysts in their mullerian tract.
- Alterations in interactions between granulosa cells and the cell of origin of ovarian epithelial tumors may be the key to familial ovarian cancer predisposition in individuals carrying germline BRCA1 mutations.

SIGNIFICANCE

- A better understanding of the normal interactions between ovarian granulosa cells and the cell of origin of ovarian epithelial tumors may lead to novel strategies for the identification of individuals at risk and for ovarian cancer prevention.
- The results support the hypothesis that ovarian epithelial tumors are of mullerian origin, which is important for the understanding of their precursor lesions and for the development of effective screening strategies for their early detection.

ACKNOWLEDGEMENTS

- Rajas Chodankar, Hai-Yun (Helen) Yen, Hao Hong, Sepideh Karimi (Dubeau Lab)
- Stanford Kwang, Hai-Yun Yen (Maxson lab)
- Frank Sangiorgi (USC/UCLA)
- Axel Schönthal (USC)
- French Anderson (USC)
- Nori Kasahara (USC/UCLA)
- Chu-Xia Deng (NIH)
Proteomic discovery of biomarkers for prognosis in ovarian cancer

Eric T Fung

Proteomics, defined
- The study of the expression, structure and function of proteins, and the interactions between proteins.
  - Where and when are proteins expressed? Abundance?
  - Protein modifications and activities
  - Interactions: Protein-protein, protein-DNA, protein-small molecule, etc
  - Protein structure
- It represents the protein counterpart to the analysis of gene function.
- Initial goal was to rapidly identify all the proteins expressed by a cell or tissue – a goal that has yet to be achieved for any species

Proteomics vs Genomics
- Proteins actually do the work of the cell
  - DNA/RNA analysis cannot predict the amount of a gene product made (of and when)
  - RNA quantitation does not always reflect corresponding protein levels
- Genomics cannot predict post-translational modifications and the effects thereof
  - Post-translational modification is extensive: so far more than 200 different types of modifications have been reported! How does modification alter protein function?
  - ~30,000 human genes yields 1,200,000+ protein variants?
  - Multiple proteins can be obtained from each gene (alternative splicing, RNA editing)

Protein biomarkers
- Differentially expressed proteins may serve as indicators or markers of a phenotypically altered state.
- Protein marker assays are employed to:
  - Detect a variety of disease states
  - Track severity of disease
  - Monitor response to drug treatments
- The utility of protein biomarkers benefits
  - Pharmaceutical discovery research
  - Preclinical toxicology
  - Basic and clinical research
  - Clinical development
  - Diagnostics

Translational proteomics process

Pattern Track™ workflow
Study design

- Flexibility in the study design
- Clinical question not limited by the technology

Three basic rules

- **Rule #1. Know what you're looking for.**
  - Broad questions require more samples to have clinical utility
  - Minimize sample variability
  - Control for all relevant conditions
- **Rule #2: Avoid systematic biases.**
  - Pre-analytical biases
  - Analytical biases
- **Rule #3: Don't misuse statistics.**
  - Feature selection
  - Independent validation

The clinical question

- Clear-cut clinical question
- Unmet clinical need
- The marker will affect patient management and patient outcome
- Know the desired results
- Controls just as important as disease samples

Designing the biomarker discovery project

- Define the clinical question
- Establish success criteria or statistical measurements of assay success
- Determine sample size for pilot and validation studies
- Carefully select control samples
- Establish standard sample collection and storage procedures

The two languages of clinical proteomics

- **Clinical**
  - Clinical question
  - Clinical trial design
  - Clinical specificity, sensitivity
  - Positive/negative predictive value
- **Analytical**
  - Precision
  - Accuracy
  - Dynamic range
  - Analytical specificity, sensitivity

Acquire the samples

- Minimize pre-analytical biases
- Multi-institutional roster of collaborators
- Associate with clinical trials
- Retrospective first, prospective when possible
- Controls just as important as disease samples
- Get enough samples to have a statistically meaningful result
Discovery phase

Pilot study to discover multiple biomarker candidates requires:
- High sensitivity
- Increased resolving power
- Broad dynamic range

Dynamic range of the plasma proteome

Use pre-fractionation methods to access the Deep Proteome™

Equalizer Beads: Dynamic Range Compression
An Addition to the Proteomic Toolbox

Principle of Protein Equalizer Beads

Validation phase

Selection of ‘best few’ biomarker candidates
- Advanced software tools
- High-throughput capabilities
- Reproducible validation tools
Software capabilities for biomarker discovery

Importance of multiple biomarkers
Single biomarkers fail due to variations in clinical samples

Types of multivariate analysis

- **Unsupervised learning**
  - No a priori “knowledge” of groups
  - Can discover new groups/subgroups
  - Generally not useful as diagnostic algorithm
  - Examples: PCA, hierarchical clustering, k-means clustering

- **Supervised learning**
  - Class assignments required for input
  - Output is a classification (diagnostic) algorithm
  - Examples: classification trees (BPS), support vector machines, neural networks

Biostatistical framework for data modeling

- Divide discovery data into training and testing sets
- Feature selection: reduce from 1000s to <10 important variables
- Different methods of feature selection can lead to slightly different ranks of features but most important features will be common
- Further reduce number of features by calculating correlation, keeping non-correlated features
- Use selected features to create classification algorithms
- Bootstrapping/cross-validation help describe robustness of algorithms
- Choose the best algorithm and apply to validation data set

Purification and identification phase

- Purify selected biomarkers
- Use matching chromatographic resins.
- Peptide mapping and MS/MS sequencing for identification.

SELDI-Assisted Purification and ID

- Monitor purification
- Peptide mapping + MS/MS sequencing
- SDS-PAGE, Passive elution
- Protease Digestion
**Biomarker Purification**

9.3 kDa biomarker passively eluted from a 1D gel

**Biomarker Identification**

Peptide mapping and MS/MS sequencing

**Assay Phase**

Develop multi-marker assays with:
- High predictive value
- Resolving power
- Quantitation capabilities

**Affinity and chromatographic based assays**

SELDI immunoassay reveals that ITIH4 is highly processed
Total Precision Studies (NCCLS Protocol)

Top 20 peaks

Total assay:
- Normal pool = 6.77%
- Cancer pool = 7.14%

Intra assay:
- Normal pool = 12.2%
- Cancer pool = 10.9%

Inter assay:
- Normal pool = 11.2%
- Cancer pool = 10.6%

Precision of transthyretin peaks: 7-10%

Courtesy of Gordon Whiteley, SAIC

Prognosis study: Patient characteristics

- 40 patients with stage III-IV EOC ovarian cancer patients from two hospitals (Leuven and Groningen)
- Patients divided into rapid progressors (short-term) vs long-term disease free survivors based on relapse within first year
  - Short term: N=20, Median age = 65.5
  - Long term: N=20, Median age = 66
- CA125 not significantly different between groups
- Pre-op, post-op, and serial samples analyzed (171 samples total)

Sample processing flowchart

Data analysis

- Spectra were calibrated and normalized to total ion current
- 884 unique peaks across 16 conditions (4 fractions * 4 chip types)
- Peaks assessed for significance for:
  - Change in levels after surgery (using paired t-test comparing pre- and post-operative samples)
  - Predicting outcome (short- or long-term disease free survival)

Peaks with greatest difference in pre- and post-operative levels

<table>
<thead>
<tr>
<th>m/z</th>
<th>Fraction</th>
<th>Chip</th>
<th>Paired p value</th>
<th>AUC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>8350.95</td>
<td>2</td>
<td>IMAC20</td>
<td>0.0005</td>
<td>0.71338</td>
</tr>
<tr>
<td>24890.99</td>
<td>1</td>
<td>IMAC30</td>
<td>0.0006</td>
<td>0.72381</td>
</tr>
<tr>
<td>15900.05</td>
<td>1</td>
<td>IMAC30</td>
<td>0.0006</td>
<td>0.67619</td>
</tr>
<tr>
<td>6641.48</td>
<td>2</td>
<td>H50</td>
<td>0.0014</td>
<td>0.303704</td>
</tr>
<tr>
<td>19986.58</td>
<td>1</td>
<td>H50</td>
<td>0.0016</td>
<td>0.290123</td>
</tr>
<tr>
<td>8152.45</td>
<td>2</td>
<td>CIM10</td>
<td>0.0019</td>
<td>0.730965</td>
</tr>
<tr>
<td>8144.70</td>
<td>2</td>
<td>IMAC30</td>
<td>0.0019</td>
<td>0.72716</td>
</tr>
<tr>
<td>15514.24</td>
<td>1</td>
<td>IMAC30</td>
<td>0.0019</td>
<td>0.647357</td>
</tr>
<tr>
<td>13567.82</td>
<td>1</td>
<td>Q10</td>
<td>0.0020</td>
<td>0.678322</td>
</tr>
<tr>
<td>16548.60</td>
<td>1</td>
<td>IMAC30</td>
<td>0.0029</td>
<td>0.319048</td>
</tr>
</tbody>
</table>

* AUC > 0.5 indicates higher in pre-operative group i.e. levels decrease with treatment

Peak down-regulated in response to surgery

P < .001
Separation of pre- and post-treatment samples using PCA

Peaks most strongly associated with outcome (pre-treatment samples)

<table>
<thead>
<tr>
<th>m/z</th>
<th>Fraction</th>
<th>Chip</th>
<th>p value</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>11183.28</td>
<td>4</td>
<td>HS0</td>
<td>0.0011</td>
<td>0.604667</td>
</tr>
<tr>
<td>2498.41</td>
<td>1</td>
<td>HS0</td>
<td>0.0029</td>
<td>0.155556</td>
</tr>
<tr>
<td>23068.54</td>
<td>2</td>
<td>IMAC30</td>
<td>0.0034</td>
<td>0.155556</td>
</tr>
<tr>
<td>66956.90</td>
<td>2</td>
<td>IMAC30</td>
<td>0.0040</td>
<td>0.175</td>
</tr>
<tr>
<td>76482.84</td>
<td>4</td>
<td>CM10</td>
<td>0.0043</td>
<td>0.1</td>
</tr>
<tr>
<td>43124.27</td>
<td>2</td>
<td>CM10</td>
<td>0.0046</td>
<td>0.194872</td>
</tr>
</tbody>
</table>

CA125 p value is not significant.
All peaks are higher in the short time to relapse group.

Conclusions
- Novel Equalizer Bead technology may be useful in discovering and identifying novel biomarkers
- Several candidate biomarkers that correlate with surgery
- Several candidate biomarkers that correlate with outcome
- Next steps
  - Validation in larger cohort
  - Additional data analysis to determine applicability to specific clinical subgroups
  - Identification of biomarkers

Acknowledgements
- Zheng Wang
- Christine Yip
- Fujun Zhang
- Enrique Dalmasso
- Xiao-Ying Meng
- Jim Geyer
- Vanitha Thulasiraman
- Prof. Ignace Vergote
- Prof. Ate van der Zee

Scatter plots of two peaks

Separation of long and short survivors with pre-treatment samples
Application of Proteomic Technologies For Advances in Ovarian Cancer

My collaborators and I have no conflicts of interest to report. I will be speaking on the use of investigational agents and investigational use of devices.

Elise C. Kohn, MD
Head, Molecular Signaling Section
Chief, Medical Ovarian Cancer Clinic
Center for Cancer Research
National Cancer Institutes
Acting Director, Medical Education Program
National Institutes of Health

An Ideal Screening Test

- Sufficiently sensitive to detect early stage disease
- Specific enough to identify those without disease
- Easy to administer
- Effective intervention available

MOLECULAR HUNTING GROUNDS:
Where are and what are the molecular diagnostics for ovarian cancer?

DNA 40,000 genes
Comparative Genomic Hybridization (CGH) or SNP arrays

RNA 150,000 splicing events
cDNA Microarrays

PROTEIN 1.5 million post-translational processing events
Proteomics

Is serum a logical pool from which to seek and analyze biomarkers?

Blood into tumor

Blood out of tumor

What Is Proteomics?

Proteomics: The study of proteins, protein pathways and networks, and protein applications

Application:
Dissection of phenotype, genotype, activation status
Description of phenotype, genotype, activation status

Clinical Implications:
Biomarker and surrogate marker development
Clinical monitoring
Identification and characterization of therapeutic targets
Assessment and validation of molecular targeted therapeutics

Challenges of Early Stage Diagnosis

Specificity--
- Heterogeneity of cancer
- Individuality of cancer
- Uniqueness of cancer from common processes

Sensitivity--
- Small volume of premalignant or early stage lesions
- Clearance/breakdown of cancer-specific markers
- Level of host response to early stage, preinvasive disease
Ovarian Cancer

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Incidence</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Confined to ovaries</td>
<td>20%</td>
<td>90%</td>
</tr>
<tr>
<td>II</td>
<td>Confined to pelvis</td>
<td>5%</td>
<td>65%</td>
</tr>
<tr>
<td>III</td>
<td>Spread IP or nodes</td>
<td>58%</td>
<td>45%</td>
</tr>
<tr>
<td>IV</td>
<td>Distant metastases</td>
<td>17%</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>

**Serum Proteomic Pattern Diagnostics**

Serum protein signature diagnostics through mass spectrometry and bioinformatics.

**Challenges in Translating Markers to Clinical Use:**

Ovarian cancer affects 1:2500 post-menopausal women

Can you find the yellow 0?

**Specificity Critically Important**

98% specificity gives a 2% PPV (48/90 women to surgery unnecessarily to find 1 cancer)

**Surface Enhanced Laser Desorption and Ionization**

Sequential of key features

Independent validation set for blinded assessment

PELVIC MASS STUDY TO DEVELOP SERUM PROTEOMIC PROFILES FOR EPITHELIAL OVARIAN CANCER DIAGNOSIS AND PROGNOSIS

**Objective:** Prospective generation of serum proteomics pattern to detect presence of malignancy in patients with pelvic mass necessitating surgery

**Eligibility:** Pelvic mass for which a surgical diagnosis is indicated

**Study Plan:** Serum storage prior to surgical diagnosis; samples post-dx for women with diagnosed epithelial OvCa

Independent training set for algorithm(s) building

Independent validation set for blinded assessment

Sequencing of key features
NCI Proteomics Ovarian Cancer Recurrence Monitoring Prospective Trial

Objective: Prospective generation of serum proteomics pattern to detect recurrence with comparison against CA125

Eligibility:
1. First clinical remission from platin/paclitaxel therapy for EOC, fallopian tube and primary peritoneal cancers
2. Must enter within 9 weeks of completion of therapy and designation of clinical complete remission (nl exam/CA125/CT)

Study Plan: q 3mo assessment, CA-125, and research bloods, q 6mo CT
To build a repository of serial samples for proteomic and other biomarker validation

Sites: NCI, FoxChase, MSKCC, NYU, MGH, U-AB, Duko, MDA, U-Wash, Cedars Sinai, Evanston and Northwestern Univ Hosp.

Referrals: NCI Clinical Studies Support Center 1-888-624-1837

Patterns v. Biomarkers
- Patterns can be used if algorithm robust
- Identification yields insight into process of malignancy
- Process may be strong with either patterns and/or biomarkers
- Concept validated by numerous investigators with multiple cancers and other diseases

Enrichment through capture of carrier proteins
Sequencing from bound proteins yields reliable peptide identification

MALDI-QqTOF MS of Albumin-bound Peptides

SELDI-QqTOF MS of “Raw” Serum

Sequencing the Ovarian Cancer Proteome-- Where are the Leads?

Categorization of Protein Function- Stage III OvCa

Sequencing the Ovarian Cancer Proteome-- Where are the Leads?

Categorization of Protein Function- Stage I OvCa

MOLECULAR HUNTING GROUNDS:
Where are and what are the molecular targets for ovarian cancer?

DNA (45,000 genes)
RNA (150,000 splicing)
PROTEIN (1.5 million post-translational processing)
Lysate microarrays is a method for studying a large range of signals across many patients.

**Imatinib Therapy with Proteomic Profiling in Relapsed Ovarian Cancer: Stromal Therapy**

**Clinical Objective:** To determine clinical activity and toxicity profile of imatinib.

**Translational Objectives:**
1. To describe tumor cell signaling pathways and their modification by imatinib
2. To correlate signaling events with clinical outcome
3. To investigate anti-angiogenic activity of imatinib

**Eligibility Criteria:**
1. Biopsiable recurrent epithelial ovarian cancer (biopsies required)
2. No more than 4 prior treatment regimens
3. Good end organ function

**Translational Objectives**

- To describe tumor cell signaling pathways and their modification by imatinib
- To correlate signaling events with clinical outcome
- To investigate anti-angiogenic activity of imatinib

**Primary Objective:** To demonstrate biochemical modulation of EGFR, Akt, and ERK, in microdissected tumor, stroma, and skin molecular surrogates.

**Secondary Objectives:**
- Measure changes in circulating VEGF and other angiogenic cytokine concentrations and correlate with clinical outcome.
- Genotype Ras and Raf mutations; correlate with clinical events.
- Pharmacogenomics of CYP3A4/5 on sorafenib.
- Characterize pharmacokinetics.
- Measure changes in circulating VEGF and other angiogenic cytokine concentrations and correlate with clinical outcome.
- Assess biochemical changes in the Ras-Raf-MAPK and VEGF pathways.
- Safety and toxicity.

**Sorafenib (BAY 43-9006) + bevacizumab**

- **Primary Objective:**
  - Safety and toxicity
  - Assess biochemical changes in the Ras-Raf-MAPK and VEGF pathways.

- **Secondary Objectives:**
  - DCE-MRI and PET to measure tumor vascular flow; CD31 HIC
  - Characterize pharmacokinetics.
  - Pharmacogenomics of CYP3A4/5 on sorafenib.
  - Genotype Ras and Raf mutations; correlate with clinical events.
  - Measure changes in circulating VEGF and other angiogenic cytokine concentrations and correlate with clinical outcome.

- **Eligibility Criteria:**
  - All solid tumors (focused accrual in renal cell, melanoma, ovary)
  - Biopsiable disease (required for cohort 2)
  - Good end organ function
  - No limitation for prior number of therapies

**Referrals:** Clinical Studies Support Center 1-888-624-1837
Future Directions in the Development of Proteomic Advances

- Define molecular predictor(s) of presence or progression of disease
- Develop relevant diagnostic test(s)
- Apply tests for proof of principle
  - Blinded validation tests of adequate size followed by movement to randomized controlled trials
  - In vivo inhibition of the target followed by validation of target to outcome link
- Target identification of Optimal (or Effective) Biological Dose (OBD)

Thanks to my colleagues and collaborators...

Laboratory of Pathology, NCI:
- Gordon Whiteley
- Virginia Espina
- Meghan Liel
- Nana Tchabo
- Lance Liotta

Medical Ovarian Cancer Team:
- Edwin Posadas
- Virginia Kwitkowski
- Herbert Katz
- Lori Minassian
- Gisele Sarosy
- Debbie McNally

MOCRU Fellows and Nursing Staff:

And the many other colleagues whose contributions could not be listed.

Ovarian Cancer Collaborators:
- Gordon Mills
- Joe Gray
- David Fishman
- Ian Jacobs
- Niele Urban
- Jan Vermorken
- Marty McIntosh
- Gas Rodriguez
- Steven Skates
- Monica Brown Jones

Ovarian SPORES GOG

US/Italy Pharmacogenomics Consortium:
- Sergio Pescevelli
- Claudio Belluco
The Detection of Early Stage Epithelial Ovarian Cancer

David A Fishman MD, Director NCI Ovarian Cancer Early Detection Program, Director Gynecologic Oncology; Cancer Prevention and Early Detection Program New York University School of Medicine

Supported by NCI UO1CA85133, NCI P50 CA83639, NIH R01 CA89503, NIH R01CA82562, NIH R01 CA101015, NCI R21/33, NYU Cancer Institute, Greenberg Foundation, Kaleidoscope of Hope Foundation, 100 Women's Hedge Fund Foundation, SAC Foundation, NYU School of Medicine

The problem:

Epithelial Ovarian Carcinoma

- 70-75% women are diagnosed with advanced disease (as in 1960)
- Poor 5-year survival (12-15%) for advanced stage EOC
- 90% 5-year survival for stage I disease- yet often detected serendipitously
- Therefore intentional detection of early stage disease is critical

How to Detect Early Stage EOC???

- Annual CA125 and US do not achieve detection of early stage disease
- Both can provide false security or inappropriate anxiety
- Accuracy approximates 50% for early stage disease

Who is at Risk?

- Increased risk based on: personal history, family cancer pedigree, known mutation carrier, prolonged use of infertility Rx

NOCEDP Clinical Experience

- Formal Genetic evaluation and Testing
- 3D US and Microvascular Index (MVI)
- Physical examination q 6m
- Health Services, QOL, Education
- Ovarian Pap Test- outpatient 0.9 mm miniscope
- Biomarkers unique to ovarian carcinogenesis, invasion, metastasis

Clinical Risk Assessment

- Nulliparity???? (92% parous > 1 child)
- Personal and Family History- critical
- Ashkenazi descent ? (why me?)
- 38% Jewish women with ovarian carcinoma- + BRCA 1 or 2
- 20% Jewish women with premenopausal Breast carcinoma- + BRCA 1 or 2
- All affected Jewish women should be offered genetic testing

Ovarian Cancer Syndromes

- Site-specific ovarian
- Breast-Ovarian
- Lynch type II - hereditary nonpolyposis colorectal cancer (HNPCC) – 9- 12%
- Mutations of unknown significance

Risk Assessment

- Formal pedigree analysis and genetic testing and counseling by a team including board certified geneticists and gynecologic oncologists identified 581 women
- 549 BRCA1/2 +, 32 + pedigree assessment
- Prophylactic surgery consisted of a laparoscopic BSO, peritoneal washings, and comprehensive evaluation of pelvis and abdomen

Demographics

- 581 High Risk women
- 337 BRCA 1+ (58%)
- 212 BRCA 2+ (36%)
- 32 BRCA- (5.5%) yet pedigree c/w Inherited Cancer Syndrome
- Evaluation from 1990-2005
- Average Clinical follow-up 5 years

BRCA 1

- 337 women
- 7 Gynecologic malignancies
  - PPC- 2- Stage III C and IIIB
  - FT- 2- Stage IIB and IIIB
  - OVCA- 3- 1-Stage IA, 2-IIIA
- No PPC in all women s/p BSO

BRCA 2

- 212 women
- 3 Gynecologic Malignancies
  - PPC- 1 Stage IIIB
  - FT- 1 Stage IIIA
  - OVCA- 1 Stage IB
- No PPC in women s/p BSO
Cancer Detection

- 571 Benign
- 3 Primary Peritoneal Cancer - all Stage III
- 3 Fallopian Tube Cancer - Stage II/III(2)
- 4 Ovarian Cancer - 2 - Stage I, 2 - Stage III
- 10 Cancers –
  - 2 - Stage I
  - 1 - Stage II
  - 7 - Stage III

Recent Advances In Ultrasound

- Power Doppler Energy - improved specificity as secondary test (83-92%)
- 3-Dimensional volume acquisition and power Doppler - identifies architectural and vascular changes in observed mass, increases specificity from 54% to 75% as a secondary test
- Microvascular Imaging (MVI) - capillaries visualized with nanoparticles


Complex adnexal mass with multiple septations, without central flow suggestive of benign disease
serous cystadenoma - confirmed by pathology
NOCEDP

- 19,538 gynecologic U/S on 8,246 asymptomatic high-risk women (normal exam and U/S)
- 107 aberrant masses identified
- 57 surgical interventions
- 45 benign tumors, 12 cancers

NOCEDP

- 12 asymptomatic gynecologic cancers detected (4 fallopian tube, 4 primary peritoneal, 2 epithelial ovarian carcinoma, 2 uterine)
- all Stage III/IV (A, B, and C) except uterine (both stage1A G1)
- all normal US and PE 12 and 6 months prior to abnormal scan
- FT/PPC - normal ovaries

Conclusion

- US was effective in detecting asymptomatic advanced stage adnexal disease
- US is ineffective as an independent modality in the detection of early stage EOC in the high-risk population

The Future: Microvascular Imaging

- Combination of high resolution ultrasound with vascular mapping and quantification of aberrant capillary influx from pre-existing host venules stimulated by tumor neovascularization
- IV contrast agents (micro- and nanoparticles) to illuminate the extravasation associated with the influx of new “leaky” vessels
**Ovarian Pap Test**

- Minimally invasive office laparoscopy - outpatient procedure
- Genomics and proteomics can detect precancer/cancer years before cytology - Prevention

Cont Obstet Gyn 2003, NEJM 2003

**FISH assay:**

EVII and MYC for EOC detection

![FISH assay diagram](image)

**BRCA1+ Mutation with Normal Cytology**

Prophylactic BSO

Abnormal Copy Number of EVII and MYC

**Serum Proteomic Patterns**


- Identification of low molecular weight serum proteins
- MALDI-TOFO - matrix-assisted laser desorption and ionization time-of-flight
- SELDI-TOFO - surface-enhanced laser desorption and ionization time-of-flight
- Artificial Intelligence (AI) computation

![Proteomic Patterns](image)

**The Rapid Evolution of MS Instrumentation**

- Year 2002 - Low Resolution SELDI-TOF - Lancet 2002
- Year 2004 - High Resolution SELDI-TOF - ERC 2004
- Year 2005 - Ultra High Resolution Orthogonal MALDI-TOF and FT-ICR - Direct Accurate Mass Tagging Based ID - NCI 2005

**Biomarker Amplification and Harvesting by Carrier Molecules**

![Biomarker Amplification](image)
Prominent SELDI-TOF ionic species (m/z 6631.7043) identified to correlate with the presence of ovarian cancer were amplified by albumin capture.

### 456 Albumin Binding Fragments: Ovarian Cancer

- mRNA & lncRNA - LC/MS/MS
- Growth factors (p110, p60)
- Proteases (MMPs, Kallikreins)
- Proteomics- SELDI/ MALDI-TOF, ABI QqTOF, ESI-MS


What is required for the clinician and patient to achieve optimal healthcare?
Multiplexed serum assay for early detection of ovarian cancer

Anna Lokshin, PhD

OVARIAN CANCER: STATISTICS

- Fifth most common cancer in the US
- Accounts of 4% of all cancers in women
- Causes more deaths than any other gynecological cancer
- About 23,000 new cases of ovarian cancer and 14,000 deaths each year in the US
- The 5-year survival for patients with clinically advanced ovarian cancer is 15 to 20%
- The cure rate for stage I disease is usually greater than 90%
- Only 25% of all OC are found at an early stage

OUR GOAL

Develop multimarker serological test for early detection of ovarian cancer that has:

a. high specificity
b. high sensitivity
c. low cost

Luminex Assay

Comparison with ELISA

- Multiplexed quantitation of up to 100 analytes in a single sample
- Require smaller sample volume (< 50 µl)
- Are more rapid. Equilibrium is reached sooner in the near-liquid phase, so incubation times are reduced significantly, particularly if wash steps are eliminated.
- Are more sensitive. Can detect cytokines down to ~1 pg/ml.
- Cannot optimize reaction conditions for each analyte

PATIENTS

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Age</th>
<th>Histologic Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control n=85</td>
<td>Range</td>
<td>Pапillary adenocarcinoma, endometrioid</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>Carcinoma, mucinous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcinoma, poorly differentiated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenocarcinoma, serous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcinoma, clear cell</td>
</tr>
<tr>
<td>Early Stage Ovarian Cancer n=61</td>
<td>Range: 33-76</td>
<td>Median: 46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcinoma, mucinous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcinoma, poorly differentiated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenocarcinoma, serous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcinoma, clear cell</td>
</tr>
<tr>
<td>Benign Tumors n=75</td>
<td>Range: 33-88</td>
<td>Median: 46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast tumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Crystalline fibrous, serous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyst, papillary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyst, serous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyst, simple</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cystadenofibroma, serous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cystadenoma, mucinous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cystadenoma, papillary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endometrioid Fibrosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deeply invasive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myxoid benign</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malignant fibrous, serous</td>
</tr>
</tbody>
</table>

Range | Median
---|---
33-88 | 46
33-76 | 46
33-87 | 44
INITIAL SCREENING: LUMINEX ANALYTES

Panel I
- Cytokines: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p40, IL-13, IL-15, IL-17, IL-18, TNFα, TNFβ, TNFR I, TNFR II, IFNγ, GM-CSF, G-CSF
- Chemokines: RANTES, MIP-1α, MIP-1β, MCP-1, Eotaxin, MIG
- Growth and angiogenic factors: EGF, VEGF, VEGF, HGF, VEGF, HGF

Panel II
- Cancer Antigens: CA 125, CA 15-3, CEA, APP, CA 19-9
- Apoptotic proteins: sFas and sFasL
- Growth factors/Oncogenes: EGFR, Her2/neu, IL-2R, NGF, IGFs, IGFBPs
- Proteases: PSA free, Kallikreins 5, 6, 8, 11; MMP-2, 3, 7, 9, TIMP-1, 2
- Other markers: Cyfra 21-1, TPA, M-CSF, HMGB-1, S-100, LDH, CRP, osteopontin

Panel III
- Circulating antibodies against:
  - Cytokines: IL-6, IL-8
  - Growth factors/receptors: EGF, EGF, VEGF, Her2/neu, PDGF, PDGFR
  - Cancer antigens: CA 125, 15-3, 19-9, 72-4, CEA, MUC-1, PSA
  - Differentiation molecules: AFP, βhCG
  - Apoptotic molecules: survivin, Fas, FasL, transglutaminase
  - Oncogenes: c-myc, N-Ras, K-Ras, Akt1, p53
  - Cell cycle molecules: cyclin B, cyclin D

CYTOKINE PROFILES ARE CANCER-SPECIFIC

Optimal model for cytokines/CA 125

CLASSIFICATION RESULTS FOR INDIVIDUAL MARKERS
(Control vs. OC)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>%Correctly Classified</th>
<th>%Sensitivity</th>
<th>%Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>80.5</td>
<td>84.1</td>
<td>76.7</td>
</tr>
<tr>
<td>VEGF</td>
<td>73.6</td>
<td>79.5</td>
<td>67.4</td>
</tr>
<tr>
<td>MCP</td>
<td>78.2</td>
<td>84.1</td>
<td>72.1</td>
</tr>
<tr>
<td>IL-6</td>
<td>85.1</td>
<td>84.1</td>
<td>86.0</td>
</tr>
<tr>
<td>IL-8</td>
<td>79.3</td>
<td>88.6</td>
<td>69.8</td>
</tr>
<tr>
<td>IL-12</td>
<td>73.6</td>
<td>72.7</td>
<td>74.4</td>
</tr>
<tr>
<td>G-CSF</td>
<td>58.8</td>
<td>40.9</td>
<td>76.7</td>
</tr>
<tr>
<td>CA125</td>
<td>85.1</td>
<td>95.5</td>
<td>74.4</td>
</tr>
</tbody>
</table>

CLASSIFICATION RESULTS FOR OvCA vs. Control

OvCA vs. Control: CA 125 + IL-6 + IL-8 + EGFR + VEGF

OvCA vs. Benign: CA 125 + IL-6 + IL-8 + EGFR + VEGF

%Correctly Classified | OvCA vs. Control | 93.0 | 80.2 |
%Correctly Classified | OvCA vs. Benign  | 91.0 | 75.7 |

ROC curve. Early stage ovarian cancer vs. healthy controls

Area under curve = 0.966
The optimal model for circulating antibodies is shown in the table below. The classification results for OvCA vs. Control and OvCA vs. Benign are presented with high sensitivity and specificity.

### Optimal model for circulating antibodies

<table>
<thead>
<tr>
<th>CLASSIFICATION RESULTS</th>
<th>OvCA vs. Control (CA 15-3+IL-8+surv+p53+c-myc)</th>
<th>OvCA vs. Benign (CA 15-3+CEA+p53+IL-6+c-myc+bHCG+EGF+IL-8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Correctly Classified</td>
<td>97.5</td>
<td>88.0</td>
</tr>
<tr>
<td>%Sensitivity</td>
<td>94.3</td>
<td>94.3</td>
</tr>
<tr>
<td>%Specificity</td>
<td>100.0</td>
<td>79.0</td>
</tr>
</tbody>
</table>

The optimal model for cancer antigens is shown in the table below. The classification results for Cancer vs. Control (Panel of 17) and Cancer vs. Benign are presented with high sensitivity and specificity.

### Optimal model for cancer antigens

<table>
<thead>
<tr>
<th>CLASSIFICATION RESULTS</th>
<th>Cancer vs. Control (Panel of 17)</th>
<th>Cancer vs. Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Correctly Classified</td>
<td>96.3</td>
<td></td>
</tr>
<tr>
<td>%Sensitivity</td>
<td>90.0</td>
<td></td>
</tr>
<tr>
<td>%Specificity</td>
<td>99.0</td>
<td></td>
</tr>
</tbody>
</table>

---

### CONCLUSIONS I

- LabMAP technique allows for high-throughput multimarker analysis of serum markers in ovarian cancer.
- Combination of several markers allows for higher sensitivity and specificity than each single marker.
- Discovery of new marker combinations may further improve the diagnostic power of multimarker assay.

---

### Pre-clinical velocities of CA 125 and ErbB2

- CA 125 levels:
  - Control: 4758, 5644, 6790
  - Benign: 3956, 4841, 5738

- ErbB2 levels:
  - Control: 3385, 4758, 6790
  - Benign: 2362, 3385, 4798
Changes in concentrations of CA 125, EGFR and Her2/neu in women before clinical diagnosis of ovarian cancer (prospective study)

1. "0" corresponds to the point of clinical diagnosis
2. Numbers in red indicate lead time before clinical diagnosis
3. Bars indicate levels in healthy women ± SD
4. Serum samples were obtained from Dr. Ian Jacobs (University College of London, London, UK)

Velocities of cytokines and cancer markers

CONCLUSIONS II

- Decreasing longitudinal ErbB2/EGFR levels can be observed in ovarian cancer prior to clinical detection. Changes in ErbB2/EGFR levels are detectable earlier that changes in CA 125.
- Velocities of "non-specific" markers, such as cytokines and autoantibodies might be indicative of early tumorigenesis.

ACKNOWLEDGEMENTS

Lokshin Lab
Adile Marrangoni, BS
Matt Winnans, BS
Lyudmila Velikokhatnaya, MS
Bryan Nolan, MS
Ligita Grinene, MS
UPCI
Doug Landsittel, PhD
Eli Gorelik, MD, PhD
Bill Bigbee, PhD
Francesmary Modugno, PhD

University College of London, UK
Ian Jacobs, MD
Usha Menon, MD
Duke University
Jeff Marks, PhD
Variable Expression and Activity of Pharmacokinetic Variables in Ovarian Tumors

Julie A. DeLoia, Ph.D.
Associate Professor
Obstetrics, Gynecology and RS
University of Pittsburgh
Director of Research, Ovarian Cancer Center

The Problem with Current Therapy

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma conc (µg/ml) drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Therapeutic failure
Therapeutic range
Toxic levels

Patient Response to Medicine Varies

One size does not fit all... at least 30% of patients don’t benefit from some medications.

What determines drug disposition?

- Absorption - gut or blood stream → target
- Distribution - in body and in tissue
- Metabolism - liver or target tissue
- Excretion - kidneys
- Pharmacodynamics - mechanism of action

Standard of Care: Ovarian Cancer

All patients with advanced disease should receive a taxane (taxol or taxotere) and a platinum (carboplatin)

73% of patients respond
27% of patients do NOT respond

Resistant (27%)
What Determines Drug Disposition in Ovarian Cancer Patients?

Carboplatin: Renal Clearance, DNA Repair Enzymes
Paclitaxel: MDR-1, CYP2C8
Docetaxel: MDR-1, CYP3A4, CYP3A5

The Human ATP-Binding Cassette (ABC) Transporter Superfamily

Largest family of TM proteins, 48 members
Genetic variation results in human disease (CF)
Overexpression can lead to drug resistance!
ABCB1, MDR-1, Pgp-1
ABCC1, MRP1
ABCG2, BCRP, MXR, ABCP

Cytochrome P450 Enzymes
- Superfamily of Phase I enzymes
- > 55 genes in family
- Large inter-patient differences
- Genetic basis is not well understood
- Ethnic differences in expression
- Liver and kidney are major sites
- Many epithelial tumors also express

How much variation is there?

<table>
<thead>
<tr>
<th>Gene</th>
<th>PM</th>
<th>IM</th>
<th>EM</th>
<th>UM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6</td>
<td>10%</td>
<td>35%</td>
<td>48%</td>
<td>7%</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>4%</td>
<td>36%</td>
<td>58%</td>
<td>N/A</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>3-21%</td>
<td>N/A</td>
<td>79%</td>
<td>97%</td>
</tr>
</tbody>
</table>

PM = poor metabolizer
IM = intermediate metabolizers
EM = extensive metabolizers
UM = ultrametabolizers

The typical ABC-transporter consists of 4 domains, 2 highly hydrophobic membrane-spanning domains, which form the translocation pathway, and 2 peripheral membrane domains, which couple ATP hydrolysis to the transport process.
Do Ovarian Cancers Express PK-related Genes?

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR-1</td>
<td>47/48 (98%)</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>33/48 (69%)</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>4/48 (8.3%)</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>42/47 (89%)</td>
</tr>
</tbody>
</table>

Expression Levels Vary Widely

<table>
<thead>
<tr>
<th>Gene</th>
<th>Relative Gene Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR-1</td>
<td>0 0.5 1 1.5 2 2.5 3 3.5</td>
</tr>
<tr>
<td>CYP2C8</td>
<td></td>
</tr>
<tr>
<td>CYP3A5</td>
<td></td>
</tr>
</tbody>
</table>

Relationship between expression and grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>MDR-1</th>
<th>CYP2C8</th>
<th>CYP3A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 (N=10)</td>
<td>221 +/- 436</td>
<td>13.8 +/- 15.3</td>
<td>108 +/- 97</td>
</tr>
<tr>
<td>Grade 2/3 (N=26)</td>
<td>634 +/- 2010</td>
<td>56.6 +/- 129</td>
<td>447 +/- 1159</td>
</tr>
</tbody>
</table>

Relationship between expression and stage

<table>
<thead>
<tr>
<th>Stage</th>
<th>MDR-1</th>
<th>CYP2C8</th>
<th>CYP3A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1 (N=10)</td>
<td>119 +/- 139</td>
<td>14.5 +/- 15</td>
<td>201 +/- 343</td>
</tr>
<tr>
<td>Stage 2/3 (N=26)</td>
<td>290 +/- 481</td>
<td>56.6 +/- 129</td>
<td>435 +/- 1158</td>
</tr>
</tbody>
</table>

Relationship between expression and histologic type

<table>
<thead>
<tr>
<th>Histologic Type</th>
<th>MDR-1</th>
<th>CYP2C8</th>
<th>CYP3A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometroid (N=8)</td>
<td>179 +/- 155</td>
<td>19.5 +/- 24.5</td>
<td>311 +/- 1955</td>
</tr>
<tr>
<td>Others (N=28)</td>
<td>260 +/- 468</td>
<td>52 +/- 125</td>
<td>305 +/- 718</td>
</tr>
<tr>
<td>Papillary serous (N=18)</td>
<td>269 +/- 454</td>
<td>74 +/- 132</td>
<td>317 +/- 832</td>
</tr>
<tr>
<td>Others (N=18)</td>
<td>214 +/- 393</td>
<td>46 +/- 19.9</td>
<td>484 +/- 1211</td>
</tr>
</tbody>
</table>

Metabolism Does Occur in Tumor Cells
Rate of metabolism relative to expression

\[ R^2 = 0.94 \]

Do levels predict chemosensitivity?

**Transporters:**
- ABCC1
- ABCB1 (MDR-1)
- ABCG2

**Cytochrome P450:**
- CYP2C8
- CYP3A4
- CYP3A5

**Drugs:**
- Taxol
- Taxotere
- Etoposide, Topotecan
- Gemcitabine, 5-FU

CYP2C8 expression correlates with response to paclitaxel

**CYP3A5 expression does not correlate with response to docetaxel**

**MDR-1 expression does not correlate with response to docetaxel or paclitaxel**
Other Transporters - ABCC1 (MRP1)

No significance

Other Transporters - ABCG2 (BCRP)

Significant only at the extreme

Where are we going?

Long Term Aims:

- Individualized Doses: genotype/phenotype - drug selection
- Predict Adverse Events - before they happen
- Decrease Toxicity - therapeutic dose/patient
  
  Decrease Morbidity, Mortality, Cost

Aventis Phase II Trial: carboplatin and DOC

1. Can we correlate genotype, phenotype, PK?
2. Can we alter drug dose based on #1?

Outcome variables: metabolites, ADR, tumor response, DFI

Retrospective Trial: Platinum resistance

ERCC1 common variant

Retrospective: GOG
Prospective: MWH
## Acknowledgements

**MWRU**
- Jackie Jones-Laughner
- Janiene Patterson
- Mary Strange

**UPCI**
- William Zamboni, Ph.D.
- Sandra Strychor

**Precision Therapeutics Inc.**
- Holly H. Gallion, M.D.

**MWH**
- Joseph Kelley, M.D.
- Bob Edwards, M.D.
- Panji Sumikkumvanich, M.D.
- Tom Krivak, M.D.

**Washington Univ.**
- Howard McCleod, Ph.D.
- Sharon Marsh, Ph.D.

**Financial Support:**
- Scaife Family Foundation
- The Pittsburgh Foundation
- Aventis Pharmaceuticals

## Questions?
The Challenges of Reducing Ovarian Cancer Mortality

Karen Johnson
Division of Cancer Prevention
National Cancer Institute
NIH, DHHS
October 25, 2005

Reducing Ovarian Cancer Mortality
DISCUSSION PLAN

- Dimensions of the challenge
- Pioneers
- Milestones
- Tools
- Questions
- Some personal observations

Reducing Ovarian Cancer Mortality
DIMENSIONS: STAKEHOLDERS

- Survivors
- Women at risk
- Men who share the burden

Reducing Ovarian Cancer Mortality
2005 USA STATISTICS

- Incidence 22,220
- Mortality 16,210

Jemal et al., CA Cancer J Clin 2005

Reducing Ovarian Cancer Mortality
PIONEERS: DR. ROBERT SCULLY

- Professor, Harvard Medical School
- 1958: “Endocrine Pathology of the Ovary”
- 1973: Architect of World Health Organization’s classification of ovarian tumors
- An expert in ovarian cancer precursors
  - “…epithelial inclusion cysts have a greater propensity to undergo neoplasia than does the surface epithelium itself.”
  - “…most epithelial ovarian tumors are intraparenchymal, rather than being located on the ovarian surface.”

Reducing Ovarian Cancer Mortality
PIONEERS: DR. NELLY AUERSPERG

- Professor of Obstetrics and Gynaecology, University of British Columbia, Vancouver
- 1954: AOA
- 1962: JNCI report of cell cultures from carcinomas
- First to isolate, characterize and develop culture methods for OSE
  - Growth, differentiation, and apoptosis in culture
  - Influence of growth factors and hormones
  - Transformation by sequential gene transfection
Reducing Ovarian Cancer Mortality
PIONEERS: DR. ALICE WHITTEMORE

- Professor of Epidemiology and Biostatistics, Stanford University
- Institute of Medicine, National Academy of Sciences
- The Collaborative Ovarian Cancer Group
- Defining the impact of pregnancy and oral contraceptives on ovarian cancer rates
- “…despite the high risks of cancer of the breast and ovary among BRCA1 and BRCA2 mutation carriers, some 30% of these women are estimated to reach age 70 years without developing either cancer.”
- Home page: http://www.stanford.edu/~alicesw/

Reducing Ovarian Cancer Mortality
MILESTONES: DEBULKING

- 1994: NIH Consensus Development Conference Statement
- 2002: Meta-analysis, effect of cytoreduction with universal primary platinum-based therapy
- 2005: As a consideration for intraperitoneal therapy

Reducing Ovarian Cancer Mortality
MILESTONES: PLATINUM

- 1978: Cisplatin approved by FDA
- 1989: Approval of carboplatin

Reducing Ovarian Cancer Mortality
MILESTONES: TAXOL

1998: Paclitaxel approved by FDA

Reducing Ovarian Cancer Mortality
STRATEGIES: SINGLE AGENT

- ICON3
- GOG 132
- Agents

Reducing Ovarian Cancer Mortality
STRATEGIES: WEEKLY THERAPY

- Agents
- Advantages
Reducing Ovarian Cancer Mortality

STRATEGIES: MICROENVIRONMENT

GOG 170D
- Reported at ASCO 2005, Abstract 5009
- Bevacizumab for persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer
- 15 mg/kg IV q 3 weeks until progression or prohibitive toxicity
- 62 recipients: 3CR, 8PR, 34SD

Reducing Ovarian Cancer Mortality

QUESTIONS

- How does the disease progress in spite of promising treatments?
- How do we get beyond the surface?
- How can we exploit the hormonal nature of this disease?

Reducing Ovarian Cancer Mortality

QUESTIONS

- How does the disease progress in spite of promising treatments?
- How do we get beyond the surface?
- How can we exploit the hormonal nature of this disease?

Reducing Ovarian Cancer Mortality

GEOGRAPHIC VARIATION

The Gambia
India
China
Japan
France
Israel
Canada
UK
Norway
Sweden
Germany
USA

Age-adjusted incidence of ovarian cancer (per 100,000 women)


Reducing Ovarian Cancer Mortality

HORMONES: RISK MODIFICATION

- Pregnancy
- Oral contraceptive exposure

Reducing Ovarian Cancer Mortality

HORMONES: RISK MODIFICATION

- Pregnancy
- Oral contraceptive exposure

Reducing Ovarian Cancer Mortality

PARITY

- With first full term pregnancy, risk reduction on the order of 40%
- Modeled risk reduction of 14% for each succeeding pregnancy
- Can apparent protective effect be simulated?

Whittemore: Am J Epidemiol 136:1184

Reducing Ovarian Cancer Mortality

PARITY

- With first full term pregnancy, risk reduction on the order of 40%
- Modeled risk reduction of 14% for each succeeding pregnancy
- Can apparent protective effect be simulated?

Whittemore: Am J Epidemiol 136:1184

Reducing Ovarian Cancer Mortality

INFLUENCE OF A SINGLE PREGNANCY


Reducing Ovarian Cancer Mortality

INFLUENCE OF A SINGLE PREGNANCY

### Reducing Ovarian Cancer Mortality

**ORAL CONTRACEPTIVES**

- Risk reduction around 30% for ever use, about 10% for 1 year or less vs. 60% for 6 or more years
- Consistency across studies
- Gradient in risk reduction related to duration of use and potency of progestin
- Biologic plausibility: macaque study

Whitemore, Am J Epidemiol 136:1184

### Reducing Ovarian Cancer Mortality

**PARITY AND ORAL CONTRACEPTIVES**

<table>
<thead>
<tr>
<th>Parity</th>
<th>Years of OC Use</th>
<th>Relative Risk of Ovarian Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>0.41</td>
</tr>
<tr>
<td>0/3</td>
<td>0.41</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Hartge et al., Obstet Gynec 1994; 84:760
Beyond Treating the Patient

Patricia Goldman
Ovarian Cancer National Alliance

Ovarian Cancer National Alliance

• Umbrella Organization
• Thousands of Women
• Our Voices united
• Our Hope Alive
• Advocacy
• Education
• Awareness

ADVOCACY

• Develop and implement strategies at Congressional and Federal Agency level
  – Department of Defense
  – National Cancer Institute
  – Food and Drug Agency
  – Centers for Disease Control

  Related issues – Reimbursement; medicare

ADVOCACY

• Train grassroots advocacy leaders
• Mobilize grassroots
• Work in Coalition
  – Society of Gynecologic Oncologists
  – WeCAN
  – Cancer Leadership Council
  – Partner Members

  NOCC

EDUCATION

• Survivors Teaching Students
• TEAL Training
• Annual Conferences
• Outreach
• Fact Sheets
• Newsletters

AWARENESS

Turn Up the Volume Campaign
Address the “Worried Well”
Placement of stories in the media
Website: http://www.ovariancancer.org
Quilts
Partnerships

• Partnership with the research and patient care communities
• Publicize the importance of treatment by a gynecologic oncologist
• Make patient population aware of the value and availability of clinical trials
• Recommend qualified advocates for Institutional Review Boards
• Recommend qualified advocates for federal panels and other review committees
Alone

They wait with me as I wait for news.
I hear what is said, but think it can't be true.
Knowing alone I must go through.

They wait with me as treatments begin, never knowing when I may give in, give up the battle I am in.

Alone I face the end results, alone to wonder with my thoughts.

Alone at the end we all must be, old friends and family to welcome me.
Guiding me to a brand new life.

~~ Cyndee DePastino ~~
Psycho-social Issues in Diagnosis and Recurrence: Effects on Patients and Their Families

Heidi Donovan, PhD, RN
Univ. of Pittsburgh School of Nursing

I. Overview

II. Why measure Psycho-social Issues?
   a. As an endpoint for evaluating treatment outcomes
   b. As a predictor of treatment response
   c. To identify rehabilitation/support needs
   d. To identify factors related to high quality of life so that we can design interventions and target them to those who need it most.

III. Evaluating response to treatment
   a. Women with progressive/recurrent disease
   b. After 2 cycles:
      i. 7/27 (26%) “objective” responders
      ii. QOL improvements (esp functional and emotional) in 41-48%
      iii. 52% reported improvements in pain control
      iv. Improvements lasting, on average 2-3 months.

IV. As a predictor of treatment response
   a. QOL scores when beginning treatment associated with survival.
   b. Cognitive, Emotional, Physical, and Role Function predicted 12 month survival in 81% - 85% of cases
   c. Group of predictors equal to stage, age at diagnosis, residual disease, and recurrent disease.

V. Which Psycho-social issues do we measure? How do we measure them? How do we improve them?

VI. PMBC Model
   a. Chronic/Stable Burdens & Resources
      i. Demographics
      ii. Personal Attributes
      iii. Social/Environmental Attributes
   b. Psychological Pathways
   c. Behavioral Pathways
   d. Biological Pathways

VII. Evidence in Oncology

VIII. Evidence in Ovarian Cancer
IX. Specific Groups at Risk:
   a. Poor Performance Status
   b. Younger women
   c. Women with young children

X. Critical Times for Intervention:
   a. At diagnosis
   b. Treatment
   c. At the end of initial treatment
   d. Remission
      i. Survival Guilt
   e. Recurrence
   f. Long-Term Survivors
      i. Don’t assume that long-term survivors have regained previous levels of well-being.
      ii. NOCC Survey of Long-term survivors
          1. 20% still experience long-term treatment side effects
          2. 43% would still like to participate in a support program if one were available
          3. Spiritual well-being was associated with mental and physical health status.
          4. 6% met cutoff scores for depression

XI. Targets for intervention
   a. Depression
      i. Screen for depression:
      ii. Immediately after diagnosis and recurrence are very high risk times
      iii. Treat with anti-depressants and counseling
      iv. Most will make accommodations to their perceptions of QOL after the crisis period.

   b. Control symptoms!
      i. Most significant symptoms reported by women at different phases of disease trajectory (n=713):
      ii. New Projects: NINR funded “Internet-Based Cancer Symptom Management: WRITE symptoms
         1. Timeframe 6-9 months:
         2. Private message boards linked to NOCC web site
         3. Facilitated by a nurse
         4. Focus on symptom management
         5. Coping with symptoms and side effects of disease and treatment
         6. Emotional expression and support and guidance
XII. Emphases on broad definition of “Well-Being”
   a. Autonomy
   b. Environmental mastery
   c. Personal Growth
   d. Positive Relations with others
   e. Purpose in life
   f. Self-Acceptance

XIII. Types of Support from HCP’s
   a. Education/Informational Support!! What to expect… resources… side effect management… coping…
      i. More emphases on timing of education
      ii. What they can hear when they can hear it.
   b. Support: support groups… counseling… caregiver support… spiritual support…
      i. NOCC:
         1. On-line discussion boards
         2. PUP program
         3. Opportunity for advocacy, supporting others, future benefit

XIV. Social support and Disclosure
   a. Improve social support through enhancing EXISTING supports
   b. Family training/support

XV. Supporting the Family: Special Issues

XVI. Future Directions in Research & Practice
Background to IP Bioimmunotherapy

- Both bioimmunotherapy and chemotherapy agents delivered IP since the late 1960’s
- R.B. Jones, S. Howell, V. DeVita, M. Markman, R. Dedrick & M. Flessner
  Major contributors to IP delivery methodology and pharmacokinetics
- Sensitivity of ovarian cancer to systemic chemotherapy led to IP studies to achieve elevated intratumor drug concentrations — provided incentive for IP biotherapeutics

POSITIVE RANDOMIZED INTRAPERITONEAL CHEMOTHERAPY TRIALS


IP Bioimmunotherapy - Goal

- Inhibit tumor growth in the abdominal cavity

By exposing tumor and/or immune cells to “biologically effective” concentrations of agents with selective targeting properties

Prior IP Bioimmunotherapy Studies

| C. Parvum | Webb, Mantovani, Bast (1978-83) |
| rIFNα | Berek, Wilkens, Frasier (1985-90) |
| Viral oncolysate | Freedman (1984-89) |
| rIFNg+CDDP/Carbo | Nardi, Berek, Markman, Frasci (1998-94) |
| rIL2 | D’Aquisto (1988) |
| rIL2+LAK | Chapman, Melioli, Edwards (1988-97) |
| Bifunctional a/b+rIL2+ T-cells | Stewart, Stein (1990) |
| rIL2+TIL | Canevari (1995) |

Freedman
Response Profile of IP Bioimmunotherapy

**Responses:**
- Mostly in tumors < 1 cm
  - rIFNα ± chemo in chemosensitive pts.
  - rIL2 ± LAK or ± αCD3-folate Rc
  - not possible to separate IL2 effect
  - rIFNg – 23% CPR (incl. tumors > 1cm)

**Clinical Toxicity:**
Generally acceptable – dose/schedule dependent

from P. Hwu & R. Freedman, J Immunother, 2002

Problem Areas for IP Therapy

➢ IP catheter issues:
  - May require operative procedure – cost considerations
  - Blockage – require replacement
  - Trauma to bowel
  - Infection - frequency varies (< 20%)
  - Tumor implants at port or catheter site (< 2%)
  - Adhesions - preclude optimum delivery

Problem Areas for IP Therapy

➢ Non-invasive method to evaluate response
  - Laparoscopy/laparotomy — current method for MRD – FDG-PET

➢ Limited and variable penetration of drugs
  (Dedrick, Flessner, JNCI, 1997; J of Controlled Release, 1998)

Pharmacokinetics (PK) & Pharmacodynamics (PD) in IP Trials

➢ Studies showing limited penetration of drugs by diffusion (e.g., small molecules m.w.< 1000) or by convection (large molecules, i.e. antibodies)

➢ Study by Flessner et al also demonstrated elevated intratumoral pressures greater than maximum feasible intraabdominal pressure

  □ Tannock IF et al, Clin Ca Res, March 2002
  □ Flessner M et al, Clin Ca Res, April 2005

Pharmacokinetics (PK) & Pharmacodynamics (PD) in IP Trials

➢ Include PK & PD in all phase I & II trials

➢ Cytokine levels (drug induced & endog.) in blood and peritoneal fluid can guide dose/schedule, and possibly predict ADRs

➢ Identify and monitor markers of activation (transcripts/cell surface proteins) or apoptosis

➢ Contribute to future treatment designs
Immunopharmacology and Cytokine Production of a Low-Dose Schedule of Intraperitoneally Administered Human Recombinant Interleukin-2 ... R. Freedman, et al, J Immunotherapy, 1997

- IP rIL2 — 600,000 IU/m^2 Day 1-4 (bolus)
  * Day 1 → IL10 ↑ in 4/4 patients
  * IFNγ ↑ in 1/4 patients
  * Day 4 → IL10 ↑ in 4/4 patients
  * IFNγ ↑ in 4/4 patients

- Conclusions regarding IL10:
  * Enhanced by IP IL2
  * Does not prevent IFNγ production
  * May interfere with T cell activation in vivo

Cytokine/Chemokine Transcripts in a Responding EOC Patient

Quantitative measurement of cytokine/chemokine transcripts in RNA extracts of peritoneal exudate cells (PEC)

<table>
<thead>
<tr>
<th>Cytokine/Chemokine</th>
<th>Pretreatment (agm/l)</th>
<th>Posttreatment (agm/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 3</td>
</tr>
<tr>
<td>IFNγ</td>
<td>&lt; 2,470</td>
<td>38,660</td>
</tr>
<tr>
<td>IL-2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-10</td>
<td>3,190</td>
<td>830</td>
</tr>
<tr>
<td>IP10</td>
<td>520</td>
<td>620</td>
</tr>
</tbody>
</table>

IL-10 Production in HLA-DR Monocytes

Proportions of MO/MA Subpopulations

ADCC & Phagocytosis

Freedman
Cytokine Production by MDM

**Supervised Cluster Analysis of Genes Differentially Expressed**

E. Wang et al., 2005

MO/MA & T-Cell Infiltration of Peritoneum

**PERITONEUM - NORMAL**

- Single mesothelial layer has stomata allowing transport of cells, proteins & drugs.
- Basement membrane
- Stroma-collagen based matrix/blood vessels, lymphatics, nerves, rare hematogenous cells.
- Glycosaminoglycans

Functions
- Protect abdominal viscera.
- Mobilize inflammatory cells to injury/infection.
**PERITONEUM-EPITHELIAL OVARIAN CANCER**

Clinical Observations Indicating Inflammation

- Hyperemia → florid appearance
- Thickening & tissue edema → ascites?
- Retroperitoneal fibrosis → mechanical obstruction, ureters, bowel, lymphatics → chylous ascites

---

**PERITONEUM- EOC continued**

Alterations That Might Precede Tumor Implants

- Inflammatory cell infiltrates
- Cytokines, growth factors/chemokines/leukotrienes produced by tumor or inflammatory cells
- Alteration adherence properties in ECs & transcapillary migration
- Reorganization of collagen matrix

---

**Dual Immune/Inflammatory Cell Effects**

**Antitumor Immunity**

- Evidence of Ag driven immune response (expansion of TIL derived T-cell lines, clones with CTL)
- Identification of Ag targets
- Correlate intratumoral TIL w/survival (Coukos, 2003)

---

**Suppression of Immunity**

- Absence of IFNγ transcript in solid EOC (Rabinowich 1996, Nash 1998)
- Reduced TCRζ expression on TIL
- Low or absent level IFNγ or IL12 in ascites
- Regulatory T cells (June) & correlates w/survival (Curiel 2004)

---

**Suppression of Immunity, p. 2**

- DR- MO/MA inhibit T cell proliferation (Loercher)
- Regulatory T cells & MO/MA produce suppressor cytokines IL10, TGFβ & IL6
- EOC tumor cells express FasL & TGFβ isotypes
- IP IL2 + IFNγ or IL12 enhance IL10 levels
- IP IL12 induces IFNγ but not IL2 (transcript & protein)

---

**Can Immune Suppression be Overcome?**

- Surgical/chemotherapy tumor debulking (P. Greenburg, Adv. Immunol., 91)
- Cytokines — IL2 reverses downregulated CD3ζ chain
- IFNγ or IL12 suppress IL10 production
- Antibodies/antisense (gene silencing) —
  - anti-TGF neutr a/b
  - anti-IL10 Rε a/b
- CD4+ CD25+ depletion—anti-CD25 mAb

What constitutes MRD?
## Potential Targets for Peritoneal & Ascitic MO/MA

<table>
<thead>
<tr>
<th>Category</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO/MA</td>
<td>Trabectedin induces apoptosis in MDM &amp; induced JMO &amp; inhibits CCL2 &amp; IL6 (Allavena et al Ca Res. 2005)</td>
</tr>
<tr>
<td>Surface Molecules</td>
<td>CD163 mediates: IL10 release (Demidora 2004)</td>
</tr>
<tr>
<td></td>
<td>MA targeted photodynamic Rx</td>
</tr>
<tr>
<td>Secreted Products</td>
<td>CXCL8 – proangiogenic &amp; increases capillary permeability (Huang S AM J. Path. 2002)</td>
</tr>
<tr>
<td>Intracellular</td>
<td>P38 MAPK – induces TNFα, IL1, IL8, COX2, Collagen I (Nobel, Science 2004)</td>
</tr>
<tr>
<td>Others</td>
<td>Vascular adhesion molecule</td>
</tr>
<tr>
<td></td>
<td>Leukotrienes</td>
</tr>
</tbody>
</table>

## Intraperitoneal Immunotherapy

**Future Challenges**

- Enhance tumor/microenvironment understanding
- Overcome defective immune cell functions
- Develop target specific agents (cells vs products)
- Improved imaging for MRD
- Improved delivery methods
- Integration with chemotherapy

## Acknowledgements

- Chris D. Platsoucas, PhD, Temple University
- Phase II IL12 Trial
- Robert Edwards, MD, University of Pittsburgh
- Michael Seiden, MD, Massachusetts General
- Carl June, MD, University of Pennsylvania

**Students**

- Bohuslav Melichar
- Amy Loercher
- Cherie Butts
- Ilyssa Okrent Gordon

**Laboratory**

- Stacie Gallardo
- Rebecca Patenia
Multi-antigen vaccines for prevention of ovarian cancer relapse. M.L. Disis, H. Gray, R.E. Swenson, A. Coveler, and L.G. Salazar, Center for Translational Medicine in Women’s Health, University of Washington, Seattle, WA. ndisis@u.washington.edu

Ovarian cancer is an immunogenic tumor and recent data indicates that an immune response directed against immunogenic proteins expressed by ovarian cancers may impact overall prognosis. Multiple tumor antigens have been identified in ovarian cancer and patients with ovarian cancer can be immunized against these antigens. The clinical behavior of ovarian cancers makes the disease particularly amenable to immune based therapies. Ovarian cancer can often be treated to a complete response with standard therapies such as surgery, radiation, and chemotherapy. Relapse after optimal standard therapy is a major therapeutic dilemma in ovarian cancer. In the majority of patients, relapse can occur months to years after the successful completion of standard therapy. Immunoconsolidation approaches, such as active immunization, may impact the outcome of patients with ovarian cancer by potentially preventing disease relapse.

Multi-antigen vaccines targeting biologically relevant proteins for preventing relapse in ovarian cancer are possible. Vaccine strategies focusing on eliciting a T-helper (Th) response may result in a productive inflammatory environment at the site of disease. Stimulating antigen specific Th immunity can, in itself, initiate a CD8+ T cell response, provide help for the expansion and augmentation of low level pre-existent tumor antigen specific immunity, impart long lasting memory, and is associated with epitope spreading.

Increasing the tumor specific T cell response in vivo via active immunization may also serve as a platform for the development of additional immune based strategies for the treatment of ovarian cancer. Tumor competent T cells can be expanded ex vivo more readily from vaccinated as compared to vaccine naïve women with ovarian cancer. The ability to expand T cells ex vivo to large numbers allows the potential infusion of tumor specific T cells for therapeutic purpose. Adoptive T cell therapy can increase the numbers of tumor specific T cells in vivo to greater levels than can be achieved with vaccination alone. Such a robust response may be needed to eradicate minimal residual ovarian cancer remaining after standard therapy.
"Immunobiology of MUC1 tumor antigen: lessons learned and future implications in ovarian cancer"

Anda Vlad MD, PhD
Department of Immunology
University of Pittsburgh
School of Medicine

MUC1 (1989-2005)

Overexpressed on all human adenocarcinomas: ovary, endometrium, breast, pancreas, colon, lung, prostate, head and neck, etc., as well as on multiple myelomas and some B cell lymphomas

>83% of all human tumors

MUC1 = a tumor associated antigen

MUC1-Specific Immune Responses in Cancer Patients

Humoral Responses
- Mostly IgM (T-helper independent response)
- Better clinical outcome?

Cytotoxic T Lymphocyte (CTL) Responses
- MHC restricted and unrestricted CTL have been detected

Ioannides, Finn et al, J Immunol. 1993 Oct 1;151(7):3693-703

MUC1-specific helper T cells not detected in cancer patients

Conditions promoting MUC1 immunity

↑ Anti-MUC1 Antibodies
- Mastitis
- Bone Fracture
- IUD Use
- Current Smoking
- Pelvic Surgery

Cramer, Finn et al., Cancer Epi.Biom.Prev. 2005
**Risk of Ovarian Cancer by Number of Conditions**

<table>
<thead>
<tr>
<th>Case N (%)</th>
<th>Control N (%)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 218 (57.4)</td>
<td>162 (42.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>2 220 (48.1)</td>
<td>237 (51.9)</td>
<td>0.70 (0.53, 0.93)</td>
</tr>
<tr>
<td>3 150 (45.9)</td>
<td>177 (54.1)</td>
<td>0.66 (0.49, 0.90)</td>
</tr>
<tr>
<td>4 67 (38.3)</td>
<td>108 (61.7)</td>
<td>0.51 (0.35, 0.75)</td>
</tr>
<tr>
<td>5 or more 13 (26.0)</td>
<td>37 (74.0)</td>
<td>0.30 (0.15, 0.60)</td>
</tr>
</tbody>
</table>

Cramer, Finn et al., Cancer Epi.Biom.Prev. 2005

**Anti-MUC1 Antibodies by Number of Conditions**

<table>
<thead>
<tr>
<th>Positive for Antibodies N (%)</th>
<th>Negative for Antibodies N (%)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 38 (24.2)</td>
<td>119 (75.8)</td>
<td>1.00</td>
</tr>
<tr>
<td>2 76 (32.9)</td>
<td>155 (67.1)</td>
<td>1.44 (0.91, 2.29)</td>
</tr>
<tr>
<td>3 59 (34.1)</td>
<td>114 (65.9)</td>
<td>1.51 (0.92, 2.46)</td>
</tr>
<tr>
<td>4 46 (34.0)</td>
<td>61 (67.0)</td>
<td>2.20 (1.28, 3.76)</td>
</tr>
<tr>
<td>5 or more 19 (51.4)</td>
<td>18 (48.6)</td>
<td>3.11 (1.47, 6.55)</td>
</tr>
</tbody>
</table>

Cramer, Finn et al., Cancer Epi.Biom.Prev. 2005

**Using vaccination to boost anti-MUC1 immunity**

- **In vitro**: primes CD8 and CD4 T cell responses
- **In vivo animal models**: elicits cellular and humoral immunity and tumor rejection (protection) in wild-type mice and in MUC1Tg mice
- elicits cellular and humoral immunity in chimpanzees
- elicits no autoimmunity in MUC1Tg mice or chimpanzees

**MUC1 cancer vaccine clinical trials at the University of Pittsburgh**

1. 1993-1996, Phase I in advanced pancreatic, breast and colon cancer patients who failed standard therapies (63 patients)
   - 100mer MUC1 peptide plus BCG
2. 1998-2000, Phase I pilot study in breast cancer patients following autologous stem cell transplant (4 patients)
   - 100mer MUC1 peptide plus GM-CSF
3. 2000-2002, Phase I/Ii in resected pancreatic cancer, prior to standard therapy (16 patients)
   - 100mer MUC1 peptide plus SB-AS2 adjuvant
4. 2003, Phase I/Ii in resected pancreatic cancer prior to standard therapy (12 patients)
   - 100mer MUC1 peptide loaded on DC
5. 2004, Phase I/Ii in resected pancreatic cancer prior to standard therapy (12 patients)
   - 100mer MUC1 peptide on DC
Is it possible to do better?

6. 2004, Phase I/II in resected pancreatic cancer prior to standard therapy (12 patients)
Tn100mer MUC1 peptide on PLGA or DC

\[(\text{AHGVTSPDTRPGSTAPP})_n \quad (\text{AHGVTSPDTRPGSTAPP})_n\]

GalNAc GalNAc GalNAc

100mer Tn100mer


Department of Immunology
Anti-MUC1 IgG antibody responses in DC-MUC1 vaccinated mice

ELISA plates coated with:

<table>
<thead>
<tr>
<th></th>
<th>100mer</th>
<th>Tn100mer</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC-BP-100mer</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DC-BP-Tn100mer</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Sera from mice vaccinated with:

End-point titer: 1:1,000

Summary of T cell results

<table>
<thead>
<tr>
<th>T helper responses to:</th>
<th>100mer</th>
<th>Tn100mer</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC-BP-100mer</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DC-BP-Tn100mer</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

T cells from mice vaccinated with:

Is it possible to do even better?

A relationship between inflammation and cancer

- Hashimoto's thyroiditis and thyroid cancer
- Chronic pancreatitis and pancreatic cancer
- Chronic airway inflammation and lung cancer
- Inflammatory bowel disease (IBD) and colon cancer
- Endometriosis and endometrioid ovarian cancer
Exploring the relationship between MUC1, endometriosis and endometrioid ovarian cancer

Antibody hMUC1 (IgG1) binds to both normal and hypoglycosylated MUC1. The 4H5 (IgG1) antibody is glycosylation dependent and binds preferentially to the hypoglycosylated form of MUC1. MUC1/IL10+/- mice do not develop IBD and serve as age matched controls for MUC1 expression in the absence of inflammation.

ACKNOWLEDGMENTS

Olivera (Olja) Finn, Ph.D.
John McKolanis, PhD
Kira Gantt, PhD
Pamela Beatty
Iulia Diaconu, MS

The Ovarian Cancer Research Grant
Evolution of NY-ESO-1 Vaccine Therapy for Ovarian cancer

Kunle Odunsi, M.D., Ph.D.
Attending Physician and Research Program Director
Division of Gynecologic Oncology
Roswell Park Cancer Institute
Buffalo, NY

Goals

• Does the immune system have the capacity to recognize EOC?

• Preliminary results from a phase I trial.

• Describe the repertoire of CT antigens and define potential targets for vaccine therapy.

Does the immune system have the capacity to recognize EOC?

1. The role of T cells in a mouse model of EOC

2. TIL analysis in the context of bona-fide tumor antigens in human EOC.

The critical role of T cells in EOC: syngeneic murine model using ID8

The critical role of T-cells in the control of EOC in a syngeneic murine model

CD20+, CD3+ and CD8+ TILs in ovarian cancer

Immunocompetent: survival > 120 days.
Nude (athymic) mice: survival 70 days
Prognostic significance of TILs in EOC: I

Prognostic significance of TILs in EOC: II

Prognostic significance of TILs in EOC: III

Conclusions: TILs in Epithelial Ovarian Cancer

- High Intraepithelial CD8+ TILs is associated with improved survival: > 50% improvement in overall survival (55 versus 26 months; Hazard ratio = 0.33, P=0.0003).
- High intraepithelial CD8+/CD4+ T cell ratio (>3.1) is associated with improved survival: (74 months versus 25 months; Hazard ratio 0.30, p = 0.0001).
- The unfavorable effect of CD4+ TILs is due to CD25+FOXP3+ Tregs: High versus low CD8+/Treg ratios (median 58 months versus 23 months, HR 0.31, p=0.0002).

Which antigens for vaccine therapy in EOC?

- What is the ideal cancer antigen?
  - High potential immunogenicity
  - Frequent expression in cancer & restricted expression in normal tissues
  - Essential for viability/behavior of cancer cells

- Cancer-testis (CT) antigens
  - Expression limited to germ cells of the testis.
  - Expression in malignancies in a lineage-non specific fashion.
NY-ESO-1

- Discovered by serological screening of a recombinant cDNA expression library obtained from an esophageal tumor (SEREX).
- Expression limited to germ cells and tumor cells
- Expression frequency in EOC: 43% (n = 190)

NY-ESO-1 mRNA expression

- Melanoma 35%
- Breast 30%
- Liver 30%
- Lung 25%
- Colon < 5%

NY-ESO-1

Discovered by serological screening of a recombinant cDNA expression library obtained from an esophageal tumor (SEREX).

- Expression limited to germ cells and tumor cells
- Expression frequency in EOC: 43% (n = 190)

Odunsi et al., Cancer Res. 63:6076, 2003

Prognostic significance of TILs in EOC: IV

Intraepithelial CD8+ TILs CD8+/CD4+ T cell ratio

Overall Survival (Months)

Cumulative Survival

NY-ESO-1

CD8 TIL

NY-ESO-1 (-) CD8+ TIL(-)

NY-ESO-1(-) CD8+ TIL(+)

NY-ESO-1(+) CD8+ TIL (-)

NY-ESO-1(+) CD8+ TIL(+)

Prognostic significance of TILs in EOC: IV

Intraepithelial CD8+/CD4 cell ratio

NY-ESO-1 (-) CD8+/CD4 low

NY-ESO-1 (-) CD8+/CD4 high

NY-ESO-1 (+) CD8+/CD4 low

NY-ESO-1 (+) CD8+/CD4 high

Variable presence of CD4+ CD25+ T Cells in Ovarian Tumor Infiltrating Lymphocytes

Patient BA
NY-ESO-1– Antibody– TILs ex vivo

Patient BB
NY-ESO-1+ Antibody+ TILs ex vivo

Patient CC
NY-ESO-1+ Antibody+ TILs ex vivo

Patient BA
NY-ESO-1– Antibody– TILs ex vivo

Patient BB
NY-ESO-1+ Antibody+ TILs ex vivo

Patient CC
NY-ESO-1+ Antibody+ TILs ex vivo

Patient MO
NY-ESO-1+ Antibody– TILs CD8+ ex vivo

Variable expression of GITR

Variable presence of CD4+ CD25+ T Cells in Ovarian Tumor Infiltrating Lymphocytes

Patient BA
NY-ESO-1– Antibody– TILs ex vivo

Patient BB
NY-ESO-1+ Antibody+ TILs ex vivo

Patient CC
NY-ESO-1+ Antibody+ TILs ex vivo

Patient MO
NY-ESO-1+ Antibody– TILs CD4+ ex vivo

Variable expression of GITR

Patient BA
NY-ESO-1– Antibody– TILs ex vivo

Patient BB
NY-ESO-1+ Antibody+ TILs ex vivo

Patient CC
NY-ESO-1+ Antibody+ TILs ex vivo

Patient MO
NY-ESO-1+ Antibody– TILs CD4+ ex vivo

Variable expression of GITR

Variable presence of CD4+ CD25+ T Cells in Ovarian Tumor Infiltrating Lymphocytes

NY-ESO-1 peptide vaccination prolongs survival of Tumor Bearing Host

NY-ESO-1 peptide vaccination prolongs survival of Tumor Bearing Host

EL4/HLA-A2/NY-ESO-1 cells injected IP, 35 Days Post Implant

NY-ESO-1 peptide vaccination prolongs survival of Tumor Bearing Host

0 1 2 3 4 5 100% survival

No Tumor, + Vaccination

No Tumor, - Vaccination

Tumor, - Vaccination

Tumor, + Vaccination

Days Post Tumor challenge

% survival

3
A pilot clinical trial of NY-ESO-1DP4 peptide in patients with EOC whose tumors express NY-ESO-1
Protocol RO02-28; LUD02-011

**Hypothesis:** Providing specific helper CD4+ T cells by immunization with an epitope of dual MHC class I and II specificities would lead to enhanced CTL activation and a sustained CTL response against tumor.

- Safety and toxicity
- Immune response

---

**Eligibility criteria**

- Tumor expression of NY-ESO-1 by RT-PCR and/or immunohistochemistry or LAGE-1 by RT-PCR
- HLA-DP4 (HLA-DPB1*0401 and/or DPB1*0402).
- Completion of standard chemotherapy for primary or recurrent disease and currently NED or with minimal residual disease.
- Karnofsky performance status >70%
- Life expectancy >6 months.

---

**Monitoring**

- Toxicity
  - NCI criteria
- Immunological monitoring:
  - DTH: Recall antigens, DP4, ESO1b
  - Humoral immunity
  - CD4+ and CD8+ T cell responses: Tetramer, IFN-γ ELISPOT
  - Time to disease progression.

---

**Immunization Schedule (RP02-28)**

NY-ESO-1 peptide 157-170 (ESO.DP4) 100 µg sc mixed with 0.5 mL of Montanide ISA™ (IFA)

HLA-DP4+ ovarian cancer patients with NY-ESO-1 or LAGE-1 expressing tumors

Maximum of three courses (each consisting of 5 vaccinations given at 3 weekly intervals, providing patient remains NED)

---

**Presensitization with HLA class II peptides**

PBL

Assay

Target cells: T-APC
PHA activated CD4+ T cells

Peptide from Influenza NP or from NY-ESO-1

---

**Mapping of HLA class I and II epitopes from NY-ESO-1**

Mapping of HLA class I and II epitopes from NY-ESO-1
NY-ESO-1 specific CD4+ T cell responses (IFNγ 8/14; IL5 8/9)

<table>
<thead>
<tr>
<th>Pt #7 CD4 IFNγ spots/50000 CD4+ T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eso</td>
</tr>
<tr>
<td>D22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pt #10 CD4 IFNγ spots/50000 CD4+ T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eso</td>
</tr>
<tr>
<td>D22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pt #12 CD4 IFNγ spots/50000 CD4+ T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eso</td>
</tr>
<tr>
<td>D22</td>
</tr>
</tbody>
</table>

**HLA Class I**

- HLA-A2 5 (1)
- HLA-A24 5 (1)
- HLA-A2 and A-24 1 -
- HLA-A2 or A-24 9 (2/9 = 22%)

*More time points remain to be tested*

**RP02-28: Patient Characteristics (18/18 enrolled)**

<table>
<thead>
<tr>
<th>Age (median)</th>
<th>60 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performance status</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Recurrences prior to vaccine therapy</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10 (55%)</td>
</tr>
<tr>
<td>Yes</td>
<td>8 (45%)</td>
</tr>
<tr>
<td>No of prior lines of chemotherapy</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10 (55%)</td>
</tr>
<tr>
<td>2-9</td>
<td>8 (45%)</td>
</tr>
<tr>
<td>Serous histology</td>
<td>17</td>
</tr>
<tr>
<td>Grade III</td>
<td>16</td>
</tr>
<tr>
<td>Stage of primary tumor</td>
<td></td>
</tr>
<tr>
<td>Stage III/C</td>
<td>16</td>
</tr>
<tr>
<td>Stage IIIC</td>
<td>2</td>
</tr>
</tbody>
</table>

**DTH Responses: Patient 7**

Range of ESO DTH reactions: 5-31mm in 7 patients

**Summary of clinical results**

<table>
<thead>
<tr>
<th>DTH to recall antigens at baseline</th>
<th>13 (72%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetanus</td>
<td>7</td>
</tr>
<tr>
<td>Tetanus + Candida</td>
<td>5</td>
</tr>
<tr>
<td>Candida</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease status at study entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>NED</td>
</tr>
<tr>
<td>Minimal residual disease</td>
</tr>
<tr>
<td>Non-target lesion</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NED</td>
</tr>
<tr>
<td>Progression</td>
</tr>
<tr>
<td>Recurrence</td>
</tr>
<tr>
<td>DOD</td>
</tr>
</tbody>
</table>
Patient 002: Antigen expression and TILs

NY-ESO-1 peptide vaccine induced CD4+ T cells are not able to recognize naturally processed NY-ESO-1 protein

Challenges and Lessons

- Increasing the overall level and diversity of the anti-tumor immune responses?
- Multi-antigen vaccination: which antigens?
- Protein Immunization, novel immunological adjuvants.
- Role of CD4+CD25+ T cells
- Role of IDO
- Phase II/IIb clinical trials

Repetoire of CT antigen expression in ovarian cancer (n = 100)
Phase II study of rV-NY-ESO-1 and rF-NY-ESO-1 in patients with epithelial ovarian cancer: LUD02-12

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>5</th>
<th>9</th>
<th>13</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>1</td>
<td>29</td>
<td>57</td>
<td>85</td>
<td>113</td>
</tr>
</tbody>
</table>

rV-NY-ESO-1 3.1x10⁷ PFU
rF-NY-ESO-1 7.41x10⁷ PFU

Cycle 1  V  F  F  F

Target accrual 22 patients
Safety, immunological assays, clinical assessment

Acknowledgements

- RPCI
  - Feng Qian
  - Bridget Thomas
  - Jeanine Villella
  - Kerry Rodabaugh
  - Shashikant Lele

- Ludwig Institute, NY
  - Achim Jungbluth
  - Sacha Gnajtic
  - Eiichi Sato
  - Hiroyoshi Nishikawa
  - Eric Hoffman
  - Lloyd Old
TLR-4 signaling promotes tumor growth and paclitaxel chemo-resistance in ovarian cancer

Thomas J. Rutherford
Yale University School of Medicine
2005

Inflammation → ? → Cancer

Chemo-resistance

NF-κB

Akira, 2004

TLR signaling

Akira and Takeda, 2004
A

+ C OSE R179 R182 -RT

TLR-4

500 bp -

B

EOC cells OC Tumors

+ C R182 CP70 A2780 R179 T508 T107 T197

TLR-4

88 kDa

C

(+) EOC cells (+) OC Tumors (-) EOC cells

MyD88

B-Actin

R179 R182 R452 R454 R456 T05 T16 T19

CP70 A2780 T508 T107

35 kDa

45 kDa

Cell viability (% of the control)

TIME (Hours)

100

24 h

48 h

80

0 h

GRO-alpha

MyD88(+) MyD88(-)

R182 T5 A2780 Cp70

NT LPS

MCP-1

MyD88(+) MyD88(-)

R182 T5 A2780 Cp70

NT LPS

Positive control

Negative control

Cytokine
**Acknowledgements**

<table>
<thead>
<tr>
<th>Research Staff</th>
<th>Clinical Staff</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Gil Mor</td>
<td>- Peter Schwartz</td>
</tr>
<tr>
<td>- Ayesha Alvero</td>
<td>- Masoud Azodi</td>
</tr>
<tr>
<td>- Irene Visintin</td>
<td>- Michael Kelly</td>
</tr>
<tr>
<td>- Vikki Abrahams</td>
<td>- Jessica McAlpine</td>
</tr>
<tr>
<td>- Shawn Chavez</td>
<td>- Dan Silasi</td>
</tr>
<tr>
<td>- Roy Chen</td>
<td>- Lisa Baker</td>
</tr>
<tr>
<td>- Paula Aido</td>
<td>- Martha Luther</td>
</tr>
<tr>
<td>- Serena Chen</td>
<td></td>
</tr>
</tbody>
</table>

**Diagram:**

A. Caspase 3/7 Activity (Relative Units)

- **Control Taxol (2µM)**
- **WT**
- **p-hMyD88**

B. Western Blot

- **MyD88**
- **Beta Actin**

**Diagram:**

- TLR-4
- MyD88
- Survival
- Proliferation
- Tumor-growth factors
- MCP-1, GRO, IL1,6,8, RANTES
Monoclonal antibodies specific for self antigens can trigger antigen-specific cellular immunity
- The observation represents an alternative immunotherapeutic strategy to standard “vaccine approach”
  - Review evidence from MUC1 and CA125 systems
- Consider clinical development strategies for ovarian cancer immunotherapy

MUC1-Transgenic Mouse Model to Study BrevaRex
- Developed by Dr. Gendler, Scottsdale, AZ
- C57BL/6 mice that express human MUC1 in tissue-specific fashion
- Tolerant to MUC1 on a B and T cell level
- Immunizations s.c. at weeks 0, 3, 6 and 9; 9 groups with 5 mice/group:
  - MUC1-peptide; 0.625, 1.25 and 2.5 µg/mouse
  - BrevaRex MAb-AR20.5; 50 µg/mouse
  - MUC1-peptide + BrevaRex MAb-AR20.5 complex; 0.625, 1.25, 2.5 µg of peptide + 50 µg of MAb
  - PBS
  - MUC1-peptide-KLH (PC)
- Test bleeds for antibody responses were taken at weeks 0, 4, 7 and 10
- Mice were sacrificed at week 10 and spleen cells harvested for T cell responses

Complex-Induced B Cell Responses to MUC1
Anti-MUC1 Antibody Responses

Complex-Induced T Cell Responses to MUC1
Example - CTL Induction

Clinical Study with AR20.5 (BrevaRex®)
Phase I: Dose ranging study in patients with MUC1 associated malignancy
Therapy: AR20.5 αMUC1 antibody (BrevaRex®)
Dosing: Sequential cohorts – 1 mg, 2 mg, 4 mg; 20 minute IV infusions
Endpoint: Safety
  Immune response
  Clinical outcome

Nicodemus
Summary of T cell ELISPOT data from patients treated with BrevaRex® MAb-AR20.5

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Patient No.</th>
<th>IFN-γ ELISPOT (spots/10^5 cells)</th>
<th>Baseline</th>
<th>Post-injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg</td>
<td>AA005</td>
<td>433</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>2 mg</td>
<td>AA008</td>
<td>0</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AA009</td>
<td>0</td>
<td>144</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AA111</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AA102</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AA114</td>
<td>0</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td>4 mg</td>
<td>AA106</td>
<td>140</td>
<td>800</td>
<td>1030</td>
</tr>
<tr>
<td></td>
<td>AA117</td>
<td>140</td>
<td>800</td>
<td>&gt;2800</td>
</tr>
<tr>
<td></td>
<td>AA109</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AA120</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Number of spots counted in ELISPOT assays performed after in vitro stimulation of peripheral blood lymphocytes obtained from the patients at baseline or after treatment.

**Maximum number of spots counted at any time after the first injection of MAb-AR20.5.**

---

**CA125 Antigen**

- Huge, highly-glycosylated transmembrane protein, MW >2.5 Mio Da w/o sugars, >5 Mio Da glycosylated
  - ~12,000 aa amino terminal domain
  - ~10,000 aa of 60+ repeat domains of 156 aa
  - Short cytoplasmatic tail with tyrosine phosphorylation site
- Enzymatically cleaved into extracellular matrix

---

**T Cell Epitopes on CA125**

- Full sequence evaluated for HLA-A2 epitopes
- Candidate peptides being identified
- Pentamer reagent development ongoing

---

**Monoclonal Antibody B43.13**

Oregovomab MAB-B43.13 (OvaRex®)

- Murine IgG, specific for CA125
- Formulated for 2 mg infusions (50 mL over 20 minutes)
- Complexes CA125 in circulation
- Does not have direct effects (ADCC or CDC)
- Results in altered antigen processing of CA125 and subsequent lymphocyte response

---

**CA125-B43.13 IC But Not CA125 Co-Localize with LAMP-1 6 h after Pulse**

1 h pulse with CA125-Cy3 or CA125-Cy3 + B43.13, chase for different time points, fixation and stain with anti-LAMP-1-FITC

---

**Oregovomab Complexes Increase Cellular Response with selective CD8+ (CTL) Phenotype**

Two rounds of in vitro stimulation
Study OVA-Gy-12 - Design

Patients with Recurrent Disease (n = 20)

CA125-Specific T Cells Induced with OvaRex

OVA-Gy-12

T Cell Responses Maintained in Presence of Chemotherapy

OVA-Gy-12

Advanced Ovarian Cancer

Natural History of the Disease
**Study OVA-Gy-07: Randomized Phase II Consolidation Trial**

- OvaRex 2 mg (n=73) versus placebo (n=72)
- Double-blind to treatment, immune response and serum CA125 levels
- Study run in conjunction with Canadian Study OVA-Gy-06
- Patients after completion of front-line therapy (post-surgery and chemotherapy)
  - No evidence of disease and normalized serum CA125 (<35 U/mL) prior to enrollment
  - No restriction on surgical outcome or serum CA125 level (by third cycle of front-line chemotherapy)
- Administered IV at weeks 0, 4, 8 then Q12 to relapse
- Primary Endpoint:
  - Time to disease relapse (TTR)


---

**Successful Front-Line Therapy Population**

- Small diameter residual disease (microscopic to ≤ 2 cm)
- CA125 ≤ 65 U/ml, prior to cycle 3 (Makar)
- NED
- CA125 5-35 U/ml at start of OvaRex MAb therapy

---

**Study OVA-Gy-07: Results**

Kaplan-Meier Analysis of Time to First Event (Disease Relapse or Death)

**IMPACT I/II Program**

Two Phase III Protocols; 354 Patients Total

**IMPACT I & II Enrollment to Date**

*September 2005

---

**Protocol OVA-Gy-18**

Piloting Front-Line Chemoimmunotherapy

Status: Enrolling at 7 centers
Preliminary Data 2006
OVA-Gy-18
HAMA & CA125 By Treatment Arm

Integrated Safety Experience:
Most Frequent Treatment-Emergent Adverse Events (OvaRex vs. Placebo)

<table>
<thead>
<tr>
<th>MedRA System Organ Class (SOC)</th>
<th>Frequency (%) of patients in each group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OvaRex (N=360)</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>71.4%</td>
</tr>
<tr>
<td>General disorders &amp; administration site conditions</td>
<td>54.4%</td>
</tr>
<tr>
<td>Musculoskeletal &amp; connective tissue disorders</td>
<td>51.1%</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>47.1%</td>
</tr>
<tr>
<td>Infectious and infecational</td>
<td>11.9%</td>
</tr>
<tr>
<td>Respiratory, general &amp; miscellaneous disorders</td>
<td>26.0%</td>
</tr>
<tr>
<td>Skin and subcutaneous tissue disorders</td>
<td>36.0%</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>21.1%</td>
</tr>
<tr>
<td>Reproductive system and breast disorders</td>
<td>4.0%</td>
</tr>
</tbody>
</table>

OvaRex group (N=360) combined from studies OVA-Gy-06/07, OVA-Gy-10, OVA-Gy-12, OVA-Gy-15, and OVA-Gy-16. Placebo group (N=196) combined from studies OVA-Gy-06/07 and OVA-Gy-10.

Summary
• Antibody to circulating antigen can stimulate cellular immunity
• Clinical strategy targeting minimal disease state primary development approach for oregovomab
  – Await results of IMPACT studies in early ’07
• Activity preserved with combination chemotherapy
  – Explore front-line and recurrent disease settings

Future Directions
• Identification of immune responsive patients a priori
• Coordination of pharmacologic interventions to modulate immune responsiveness
• Customization of immune modulators, cytotoxics, and specific immune stimulations is the future of cancer treatment

Acknowledgements
Research
University R&D
Joy Ciesynski Brijt Schutes L. Mary Smith
Altallex/Virexx Research
Antoine Noujaim Robert Eng
University of Pittsburgh
Theresa Whiteside Lisa Butterfield
University of Maryland
Dean Mann

Clinical Investigators
James Bearden Jonathan Benkacns
Douglas Blayney Jeffrey Boss
Patricia Brady Sandra Brooks
Richard Bubbe Linda Casson
Christina Ceder Robert Coleman
Mary J. Cunningham Susan Davison
Robert A. Barger John A. de Rosa
Robert Edwards Michael Fier
Michael Finan Neil Fincher
Francine Foss Holy Gallion

Additional Investigators
(Canada, Europe, US)
Agustin Garcia Alan N. Gordon
Don Heel Jean Hurteau
David Jinan Miaokio Kao
Joseph Kelley Stuart Lichtman
William McGuire Michael Method
Bonnie Mertens James Ohr
Gill Duck Patil Jonathan Puckett
James Roberts Daniel Smith
Peyton Taylor Joan Walker
Steven Wagner
Individualized Cancer Care

New Technology
New Era

Molecular vs. Cell Based Determinants of Resistance

DNA → RNA → Protein → Cell

Individualized Care for Cancer - A Work in Progress

- Identification of high risk women
  - BRCA1/2 (DNA)
- Accurate prognosis
- Recurrence Score (RNA)
- Prediction of response to therapy
  - Chemosensitivity and Resistance Assays (Phenotypic)
  - Targeted Therapy (IHC)
- Individualized dosing
- SNPs (DNA)

Individualized Cancer Care

- Identification of high risk women
  - 1953 - Watson/Crick double-stranded DNA
  - 1993 - BRCA1, 1994 - BRCA2
- Accurate prognosis
- Prediction of response to therapy
- Individualized dosing

Ovarian Cancer: Hereditary Risk

<table>
<thead>
<tr>
<th>Family History of Ovarian Cancer</th>
<th>Lifetime Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.5%</td>
</tr>
<tr>
<td>1 first-degree relative</td>
<td>5%</td>
</tr>
<tr>
<td>2 first-degree relatives</td>
<td>7%</td>
</tr>
<tr>
<td>Hereditary ovarian cancer syndrome</td>
<td>40%</td>
</tr>
<tr>
<td>Known BRCA1 or BRCA2 mutation</td>
<td>35-65%</td>
</tr>
</tbody>
</table>

Cancer Risk

- General Population
  - 10% Breast Cancer
  - 1.8% Ovarian Cancer
- BRCA1 mutation
  - 80% Breast
  - 20-40% Ovary
- BRCA2 mutation
  - 80% Breast
  - 10-20% Ovary
Hereditary Cancer

- Breast Cancer: 15% - 20%
- Ovarian Cancer: 5% - 10%

Recommendations for BRCA Mutation Carriers

- Monitoring
  - 18 yrs: monthly SBE, q6mo breast exam by MD
  - 25 yrs: annual mammogram/MRI, annual pelvic exam, CA125
  - 30-35 yrs: annual TVS

- Risk Reduction
  - OCPs 5 yrs: decreases OV risk by 50%
  - Tamoxifen-decrease BR CA risk

- Prevention
  - Removal of Ovaries/Tubes- 99% decrease in risk
  - Mastectomy- 90% decrease in risk

Individualized Cancer Care

- Identification of high risk women
- Accurate prognosis
  - Reserve treatment for only those most likely to benefit
  - Prediction of response to therapy
- Individualized dosing

Prognosis: Breast Cancer as an Example of Genetic Prediction

- Treatment planning based on:
  - Traditional prognostic factors
    - limited predictive power (tumor size, patient age)
    - poor reproducibility (tumor grade)
  - IHC markers (e.g., Ki-67) lacking standardization and validation

Are all patients created equally?

- Two women with breast cancer, each with the same
  - Age
  - Race
  - Performance Status
  - Stage
  - ER+, Her2 -
  - infiltrating ductal
Markedly Different Outcomes

Clinical Course: ACT neoadjuvant therapy, pCR at surgery, Tam
Outcome: NED 7 years

Clinical Course: ACT neoadjuvant therapy, progressive disease, mastectomy/radiation, Tam, Herceptin
Outcome: Local and distant recurrence at 6 months

Onco
type DX™ Technology: Candidate Gene Selection

From ~40,000 genes:

250 cancer-related candidate genes

Sources include: van't Veer et al, Nature 2002;415:530-6.

Onco
type DX™ Technology: Final Gene Set

PROLIFERATION
- Ki-67
- STK15
- Survivin
- Cyclin B1
- MYBL2

HER2
- GRB7
- HER2

ESTROGEN
- ER
- PGR
- Bcl2
- SCUBE2

GSTM1

INVASION
- CD68
- Stromelysin 3
- Cathepsin L2

SCUBE2

REFERENCE
- Beta-actin
- GAPDH
- RPLPO
- GUS
- TFRC

BAG1

Onco
type DX™ Technology: Algorithm and Recurrence Score (RS)

Recurrence Category | RS (0-100)
--- | ---
Low risk | <18
Intermediate risk | 18-30
High risk | ≥31

Onco
type DX™ Clinical Validation: Tam Only B-14 Results – DRFS

DRFS Over Time – All 668 Patients

10-year DRFS = 85%

Onco
type DX™ Clinical Validation: B-14 Results – DRFS (cont)

DRFS for the Three RS Groups

p <0.00001

Low Risk (RS <18) n = 338
Intermediate Risk (RS 18-30) n = 149
High Risk (RS ≥31) n = 181
Onco-type DX™ Clinical Validation:
B-14 Results – Overall Survival

OS for the Three RS Groups

B-14 Overall Benefit of Tam

All Patients (N = 645)

B-14 Benefit of Tam

By Recurrence Score Risk Category

Interaction p=0.06

B-20 Results

Tam vs. Tam + Chemo – All 651 Pts

B-20 Results

Tam vs. Tam + Chemo – Low Risk (RS < 18)

Tam vs. Tam + Chemo – Int Risk (RS 18–30)
**B-20 Results**

Tam vs. Tam + Chemo – High Risk (RS ≥ 31)

<table>
<thead>
<tr>
<th></th>
<th>Tam + Chemo</th>
<th>Tam p = 0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>117</td>
<td>47</td>
</tr>
<tr>
<td>Events</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>10yr</td>
<td>60%</td>
<td>88%</td>
</tr>
</tbody>
</table>

**Oncotype DX™ Clinical Validation:**

RS as Continuous Predictor

**Oncotype DX™**

- High risk/Large chemo benefit
  - Optimized chemotherapy
  - Robust markers
- Low risk/Little chemo benefit
  - Optimize local therapy and hormonal therapy

**Markedly Different Outcomes**

Low Recurrence Score  
High Recurrence Score

**Individualized Cancer Care**

- Identification of high risk women
- Accurate prognosis
- Prediction of response to therapy
- Choose therapy according to abnormalities in a particular tumor
  - Chemotherapy resistance and sensitivity assays
  - Targeted therapy
- Individualized dosing
The Challenge

- Less than one in four patients benefits from chemotherapy
- Responses are inconsistent and unpredictable from one patient to another
- Physicians are faced with an increasing number of costly therapeutic options

Selection of Cancer Treatment

- Currently based on traditional prognostic factors:
  - Age
  - Stage
  - Grade
  - Cell Type
  - IHC

Recurrent Ovarian Cancer

Treatment Options:
- Carbo
- Taxol
- Taxotere
- Carbo/Doxil
- Topotecan
- Carbo/Taxol
- Gemzar
- Gemzar/Carbo
- Gemzar/Cis
- Doxil
- Navelbine
- Hexamethymelamine

Ovarian Cancer: Recurrent Disease

Limitations of Current Trial-and-Error Approach

- Excessive or ineffective therapy results in
- Unnecessary toxicity
- Excessive costs
- Delay of effective therapy
- Emergence of resistant cells
- Individual characteristics of a patient’s particular tumor are ignored

The Need for Individualization: Platinum Resistant Ovarian Cancer

Clinical Course: 3 courses of Doxil, CA 125 drops from 2200 to 20
Outcome: NED 1 year

Clinical Course: 3 courses of Doxil, CA 125 rises from 120 pretreatment to 3289
Outcome: Pt develops ascites and pleural effusion, DOD at 6 mo.
All patients are not created equally

**Patient**
- Performance status
- Prior chemotherapy
- Immune system
- Genetic polymorphisms: Drug activation, metabolism, clearance

**Tumor**
- Drug uptake, efflux
- Drug-target interaction, activation
- Intracellular detoxification, altered expression of DNA repair enzymes

Individualized Selection of Therapy

- Chemosensitivity and Resistance Assays
  - EDR assays
  - Chemosensitivity assays (ChemoFx®)
- Targeted Therapies
  - Histological markers – which patients are eligible for new therapies

Historical CSRAs vs. ChemoFx®

<table>
<thead>
<tr>
<th>Historical Assays</th>
<th>Precision ChemoFx®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Yield</td>
<td>High Assessability &gt;90%</td>
</tr>
<tr>
<td>1-2 grams Required</td>
<td>35mg Required</td>
</tr>
<tr>
<td>Long Turn Around Time</td>
<td>10-14 days</td>
</tr>
<tr>
<td>Resistance Only</td>
<td>Sensitivity and Resistance</td>
</tr>
<tr>
<td>Non Standardized Procedures</td>
<td>Automatable, Reproducible</td>
</tr>
</tbody>
</table>

Gyn Onc Use of CSRAs

- NOCR Symposium held in conjunction with Society of Gynecological Oncologist meeting in 2003
- When presented with a case study of a recurrent ovarian cancer, 39% of attendees indicated they would recommended therapy based on a sensitivity assay

Extreme Drug Resistance Assays

- Extreme Drug Resistance (EDR) Assays
  - Tumors placed in agar
  - Exposed to a single, very high concentration of drug
  - Measures proliferation
  - Only identifies drugs likely to not work
- Examples:
  - Oncotech EDR® Assay
  - Genzyme DRA™ Assay

Chemosensitivity Assays

- Chemosensitivity (Response) Assays
  - Expose cells to increasing concentrations of chemotherapy
  - Measures cell death
  - Identifies drugs
    - won’t work
    - are likely to work
- Examples:
  - ChemoFx® Assay by Precision Therapeutics
  - EVA™ Assay by Rational Therapeutics
**ChemoResponse Calculated**

- Dose response curves are generated representing fraction of cells alive at each serial dilution.
- Increasing dose number indicates increasing concentration of drug.

**Chemotherapy Response Testing: Accuracy**

- Meta-analysis of 35 studies (n=1,603)
- Pts. receiving a drug that tested sensitive were 1.44x more likely to respond
- The RR for patients receiving a drug that tested resistant was 0.23
  - Wiesenthal, 1999

**PFS According to ChemoFx® Assay**

Prediction of Response to Therapy Received: Exact Matches, n=135

<table>
<thead>
<tr>
<th>ChemoFx® Result</th>
<th>Resistant vs. Intermediate (HR: 1.7, 95% CI: 1.2 to 2.5)</th>
<th>Resistant vs. Sensitive (HR: 2.9, 95% CI: 1.4 to 6.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS</td>
<td>9 mo for resistance, 14 mo for intermediate, not achieved in sensitive</td>
<td></td>
</tr>
</tbody>
</table>

**PFI According to ChemoFx® Assay**

Prediction of Response to Platinum Therapy (n=84)

<table>
<thead>
<tr>
<th>Response</th>
<th>Median PFS (mo)</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresponsive</td>
<td>6.95</td>
<td>4.1-9.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Responsive</td>
<td>14.26</td>
<td>10.6-19.4</td>
<td></td>
</tr>
</tbody>
</table>

No responsive vs. Responsive

<table>
<thead>
<tr>
<th>Response</th>
<th>HR: 2.38</th>
<th>95% CI: 1.23 to 4.76</th>
<th>p=0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresponsive</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Overall Survival According to ChemoFx® Assay Prediction of Response to Platinum Therapy (n=84)

<table>
<thead>
<tr>
<th></th>
<th>Median Survival (months)</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresponsive</td>
<td>20.37</td>
<td>14.3, 28.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Responsive</td>
<td>51.48</td>
<td>31.2, .</td>
<td></td>
</tr>
</tbody>
</table>

Nonresponsive vs. Responsive HR: 2.56 95% CI: 1.15 to 5.88  p=0.02

CSRAs: Evidence in Ovarian Cancer

- Von Hoff (1991)
  - ORR in IVBR was 28% vs 11% in empiric
- Kurbacher (1998)
  - ORR in IVBR was 28% vs 11% in empiric
- Ness (2001)
  - ORR with IVBR 81% vs 33% in empiric

Predictive Value vs. Other Clinical Lab Tests

<table>
<thead>
<tr>
<th>Diagnostic</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Culture &amp; Sensitivity: 1</td>
<td>60%</td>
<td>67%-96%</td>
</tr>
<tr>
<td>Predicts patient response to antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate Specific Antigen (PSA): 2,3,4</td>
<td>28%-40%</td>
<td>95%</td>
</tr>
<tr>
<td>Screening for asymptomatic prostate cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal Occult Blood Test (FOBT): 5,6,7,8</td>
<td>5%-22%</td>
<td>NA</td>
</tr>
<tr>
<td>Screening for colorectal cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steroid Hormone Receptor Status/Estrogen Receptor (ER): 9</td>
<td>77%</td>
<td>NA</td>
</tr>
<tr>
<td>Predicts which patients will benefit from antihormonal therapy-Tamoxifen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steroid Hormone Receptor Status/Progesterone Receptor (PR): 9</td>
<td>69%</td>
<td>NA</td>
</tr>
<tr>
<td>Predicts which patients will benefit from antihormonal therapy-Tamoxifen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTI's ChemoFx® Assay:10</td>
<td>64%</td>
<td>100%</td>
</tr>
</tbody>
</table>

CSRAs vs. EDR Assays

- CSRAs provide information useful to most patients
  
- EDR provides useful information only to the extremely resistant percentage of patients

Targeted Therapeutics

**Monoclonal Antibodies**
- Examples: Herceptin®, Erbitux®, Avastin®

**Small Molecule Inhibitors**
- Examples: Tarceva®, Iressa®, Gleevec®

<table>
<thead>
<tr>
<th>Targeted Therapeutics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Avastin® (bevacizumab)</strong></td>
</tr>
<tr>
<td>Target: VEGF-R (vascular endothelial growth factor receptor)</td>
</tr>
<tr>
<td>Indication: Metastatic colorectal cancer</td>
</tr>
</tbody>
</table>

| **Herceptin® (trastuzumab)** |
| Target: Her2/neu |
| Indication: Metastatic breast cancer |

| **Gleevec® (imatinib mesylate)** |
| Target: BCR-ABL and c-kit |
| Indication: CML and GIST, respectively |
Targeted Therapeutics

**Herceptin®** (trastuzumab): Her2/neu

**Indication:** Metastatic breast cancer, in combination with Paclitaxel

**Target Diagnostic:** HercepTest® (IHC), PathVysion® (FISH)

- ~25% of breast cancer patients overexpress Her2/neu
- 38% of Her2+ patients have a favorable response to Paclitaxel + Herceptin
- 15% of Her2+ patients have a favorable response to Paclitaxel alone
- When used as neoadjuvant therapy …
  - 67% of Her2+ patients have pCR to chemotherapy + Herceptin
  - 25% of Her2+ patients have pCR to chemotherapy alone

Individualized Cancer Care

- Identification of high risk women
- Accurate prognosis
- Prediction of response to therapy
- Individualized dosing
  - Pharmacokinetics
    - Pharmacogenetics

Chemotherapy Effectiveness is Highly Variable

- A 2 to 50-fold inter- and intra-individual variability with chemotherapeutic agents has been observed
- Factors impacting pK* variability:
  - Organ function
  - Genetic regulation
  - Disease states
  - Age
  - Drug-drug interactions
  - Time of drug ingestion
  - Mode of drug administration

Finding the Optimal Therapeutic Range

- Too High → Toxicity
  - Compromised immunity
- Too Low → Lack of therapeutic response
  - Continued growth of cancer

Pharmacogenetics

- SNP: single nucleotide polymorphism
  - A small genetic change, or variation, that can occur within a gene sequence
  - SNPs occur in approximately 1% of the population
- Example: CYP2D6
  - CACAGCACATCGCG
  - CACAGCACATCGTG

Pharmacogenetics

- Codeine
  - SNP in CYP2D6 → decreased activity
  - CYP2D6 converts codeine to morphine
  - Decreased conversion to morphine → decreased analgesia
- 5-Fluorouracil
  - SNP in promoter region of thymidylate synthase (TS) → protein overexpression
  - 5-FU inhibits TS to prevent cell replication
  - TS overexpression → decreased anticancer activity
**Individualized Cancer Care: A Paradigm Shift...**

- From
  - "one size fits all" trial-and-error approach
- To
  - customized cancer therapy based on each patient's unique host and tumor characteristics

**Randomized Clinical Trials**

- Opponents call for RCT comparing assay to physician selection
- Majority of cancer decisions are not based on this high of a level of evidence
  - Very few RCT comparing treatments in recurrent ovarian cancer
  - Most drugs given without this
- CSRAs are tests, not treatments
- Who is going to pay for it?
  - diagnostic tests don't have the profit margins of drug companies