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:

GRADUATE SCHOOL OF PUBLIC HEALTH
University of Pittsburgh
We're Creating a Healthier World

University of Pittsburgh
Cancer Institute

Magee-Womens Hospital
of University of Pittsburgh Medical Center

National Ovarian Cancer Coalition
Pittsburgh Division

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:

NATIONAL CANCER INSTITUTE

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

sanofi aventis

Tibotec Therapeutics
Division of Ortho Biotech Products, L.P

LHAS
Ladies Hospital Aid Society
compassion with purpose

JEWISH HEALTHCARE FOUNDATION

TELIK
Innovating for life

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

8:00 AM	**Opening Plenary** Karen Johnson, MD, PhD, MPH

Beyond Treating the Patient - Survivorship Issues

8:30 AM	**Advocacy, Patient Care, and the Research Community** Julene Fabrizio & Patricia Goldman
9:00 AM	**Ovarian Cancer: Insider Perspectives** Barbara Smith & Cynthia DePastino
9:05 AM	**Psycho-social Issues in Diagnosis and Recurrence: Effects on Patients and Their Families** Heidi Donovan, PhD
9:30 AM	**Integrating Complementary Medicine with Conventional Treatment** Maria B Yaramus, PharmD
9:55 AM	**Panel Discussion** Francesmary Modugno, PhD, MPH
10:10 AM	**Break**

New Directions in Therapeutics and Prevention I

10:30 AM	**Overview** Kristen Zorn, MD
10:35 AM	**Peritoneal Immunotherapy** Ralph S Freedman, MD, PhD
11:00 AM	**Vaccines Targeting Ovarian Cancer** Mary (Nora) Disis, MD
11:25 AM	**Immunobiology of MUC1 Tumor Antigen: Lessons Learned and Future Implications in Ovarian Cancer** Anda Vlad, MD, PhD
11:50 AM	**Evolution of NY-ESO-1 Vaccine Therapy for Ovarian Cancer** Kunle Odunsi, MD, PhD
12:15 PM	**Panel Discussion** Kristen Zorn, MD
12:30 PM	**Lunch**

New Directions in Therapeutics and Prevention II

1:30 PM	**Overview** Thomas Krivak, MD
1:35 PM	**Inflammatory Modulation of Cancer** Thomas Rutherford, MD, PhD
2:00 PM	**Oregovomab: Challenges, Lessons, & Opportunities** Christopher Nicodemus, MD
2:25 PM	**Novel Approaches for Platinum Refractive Cancer** Robert P Edwards, MD
2:50 PM	**Individualizing Cancer Therapy: Current Status, Future Directions** Holly Gallion, MD
3:15 PM	**Panel Discussion** Thomas Krivak, MD
3:30 PM	**Concluding Remarks** Francesmary Modugno, PhD, MPH

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	Joseph L Kelley, MD
Julie A DeLoia, PhD	Roberta B Ness, MD, MPH
Robert P Edwards, MD	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	Keith Durst	Claudia Lieras	Tracy Salerno
Glenn Allen	Jeffrey Eppinger	Laura Markowitz	Cheryl Schmitt
Aab Arnold	Gretchen Gierach	Kambra McConnel	Katherine Simpson
Brian Balich	Kerry Harrity	Jocelyn Moore	Bob Stoeckle
Tricina Cash	Clover Hoffacker	John Palko	Jim Swyers
Clare Collins	Alana Hudson	Amy Phillips	Robert Williams
Carey Cyphert	Shari Hutchison	Katherine Reeves	Mary Yagjian
Amy Ditta	Betty Kotowski	Joanne Rooney	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
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
Roberta 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 Pharoah
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 Whittemore
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
Division of Ortho Biotech Products, L.P.

LHAS
Ladies Hospital Aid Society
compassion with purpose

sanofi aventis

GSK Oncology

Jewish Healthcare Foundation

TELIK
Innovating for life

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

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

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

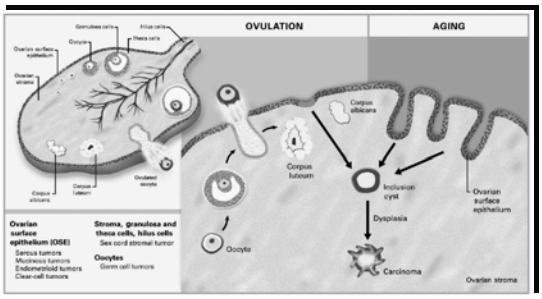
Study Objectives

- 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

Hypothetical Model of Ovarian Tumorigenesis



Drapkin and Hecht, *Women's Oncol Rev* 2002

Where does ovarian cancer arise?

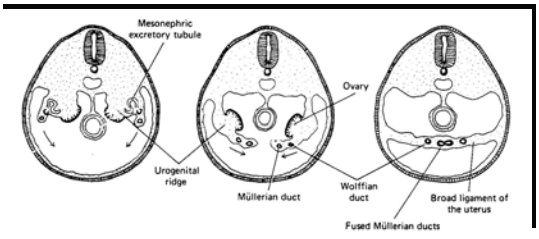
- Surface epithelium
- Morphologic alterations of surface epithelium
  - inclusion cyst
  - invagination
  - papillation
  - pseudostratification
- Secondary Müllerian system

Components of Secondary Müllerian System

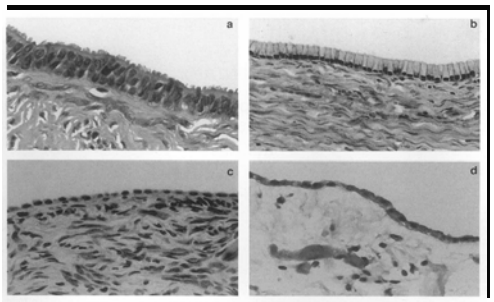
- Paraovarian/paratubal cysts
- Rete ovarii
- Endosalpingiosis
- Endometriosis
- Endomucinosis

Dubeau, *Gynecol Oncol* 1999

Urogenital Ridge (7 weeks)



Epithelial Ovarian Tumors vs. Ovarian and Peritoneal Mesothelium



Study Objectives

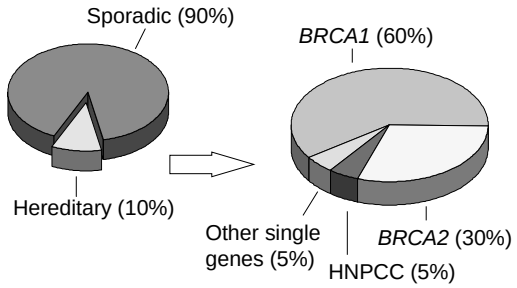
- What is the histologic origin of ovarian carcinoma?
- Is there an intermediate precursor lesion for ovarian carcinoma?

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"

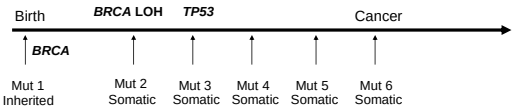
Can genetic analysis be combined with morphologic analysis to gain insight into the early natural history of ovarian carcinoma?

Genetic Causes of Hereditary Susceptibility to Ovarian Cancer

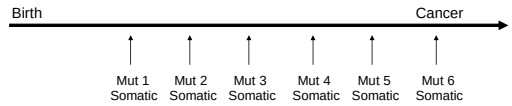


All Cancers are Genetic

"Hereditary" Cancer:



"Sporadic" Cancer:



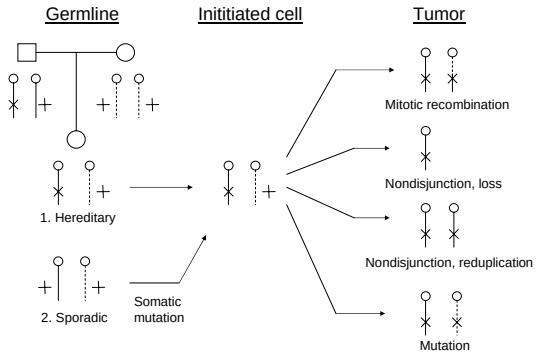
Minimal Molecular Genetic Requirements for *BRCA*-Linked Ovarian Tumorigenesis

- 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

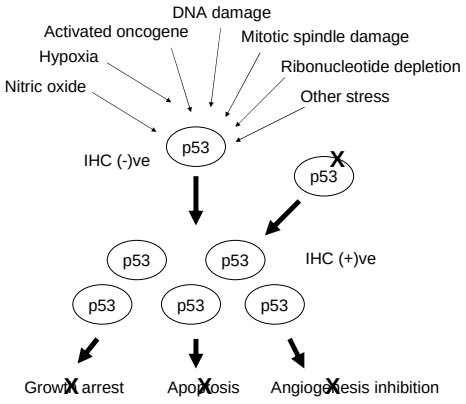
- Inheritance of mutant *BRCA* allele through germline
- Somatic loss of wild-type *BRCA* allele
- Somatic mutational inactivation of *TP53* tumor suppressor gene

Chromosomal Mechanisms for TSG Recessivity



Minimal Molecular Genetic Requirements for *BRCA*-Linked Ovarian Tumorigenesis

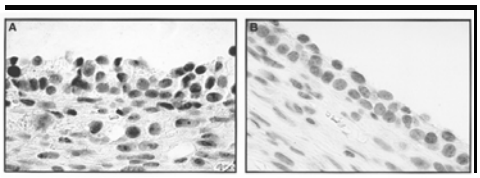
- Inheritance of mutant *BRCA* allele through germline
- Somatic loss of wild-type *BRCA* allele
- Somatic mutational inactivation of *TP53* tumor suppressor gene



Immunohistochemical Analysis of p53 Expression in Ovarian Tissues from *BRCA* Heterozygotes

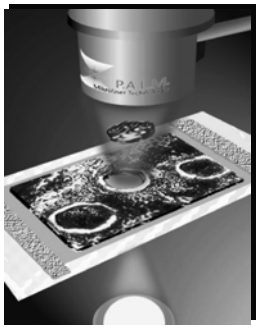
- Ovarian tissues removed prophylactically from 37 patients with a deleterious germline *BRCA* mutation
- Focal p53 expression observed in 10 (27%) of these specimens
- Invariably confined to morphologic alterations, such as cortical clefts and inclusion cysts
- Rarely observed in surface epithelium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

p53 Immunoreactivity in Ovarian Cystic Epithelium

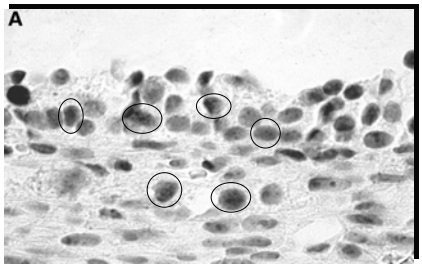


P.A.L.M. Mikrolaser Technologies

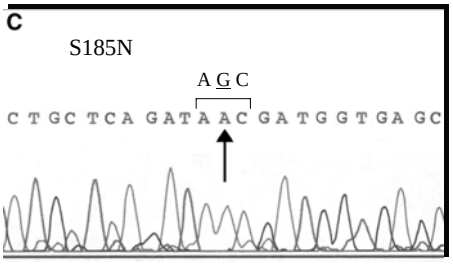
P.A.L.M. Laser Pressure Catapulting



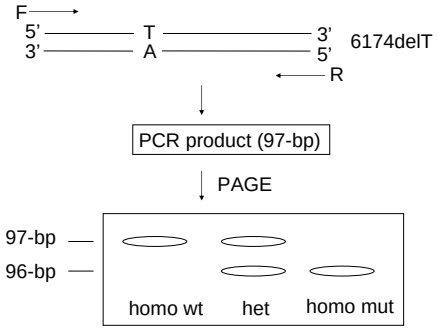
p53 Immunoreactivity in Ovarian Cystic Epithelium



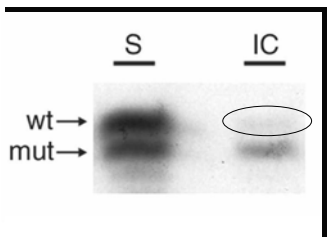
Automated Sequence Analysis of *TP53* Mutation in DNA from Microdissected Ovarian Epithelium



Detection of Mutant and Wild-type *BRCA* Alleles



Loss of Wild-type *BRCA* Allele in DNA from Microdissected Ovarian Epithelium



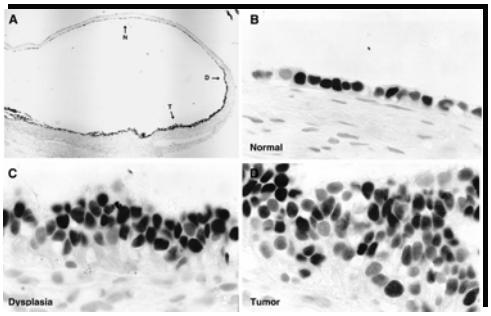
Genetic Evidence of Tumorigenic Progression in Ovarian Epithelium from *BRCA* Heterozygotes

Specimen #	*BRCA* Mutation	*BRCA* LOH	*TP53* Mutation
PO3	*BRCA1* 4050del4	Yes	H193R (CAT→CGT)
PO49	*BRCA1* 5382insC	No	H179R (CAT→CGT)
PO67	*BRCA2* 6174delT	Yes	S185N (AGC→AAC)

Stage I Ovarian Carcinomas from *BRCA* Heterozygotes

Case	Stage	Histology	Grade	*BRCA*	*TP53*
OC3	IA	Em	3	5382insC	IVS8+1
OC6	IB	PS	2	185delAG	H179R
OC7	IC	PS	2	5382insC	C275Y
OC15	IC	CC	3	185delAG	N239insT
OC16	IC	Em	2	185delAG	R282W

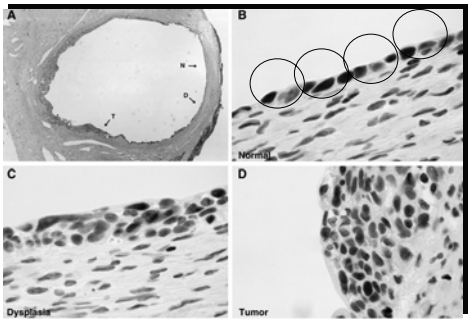
OC16



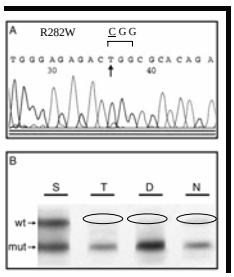
Dysplasia

- Cellular pleomorphism
- Nuclear atypia
- Loss of cellular architechtural organization
- No evidence of stromal invasion

OC6



Genetic Analysis of DNA from Microdissected Components of Stage I Ovarian Carcinoma



Stage I Ovarian Carcinomas from *BRCA* Heterozygotes

Case	Tumor		Dysplasia		Normal	
	BRCA	*TP53*	*BRCA*	*TP53*	*BRCA*	*TP53*
OC3	+	+	ND	ND	–	–
OC6	+	+	+	+	+	–
OC7	+	+	–	+	–	+
OC15	+	+	+	–	–	–
OC16	+	+	+	+	+	+

Relevance to sporadic ovarian carcinoma?

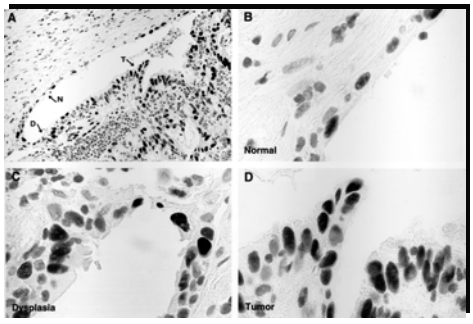
Immunohistochemical Analysis of p53 Expression in Normal Ovarian Tissues

- 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

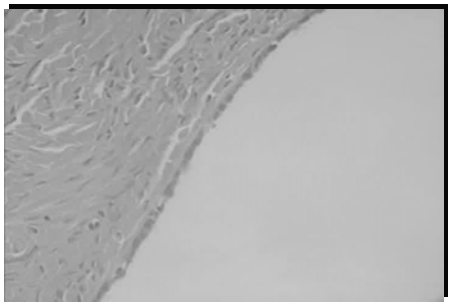
Stage I/II Sporadic Ovarian Carcinomas

- 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

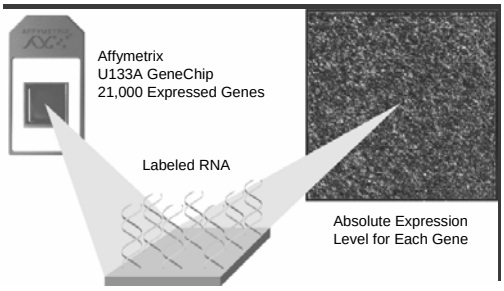
OC-7



What is the molecular phenotype of ovarian cyst vs. surface epithelium?



Oligonucleotide-Based Microarray for Gene Expression Profiling



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?

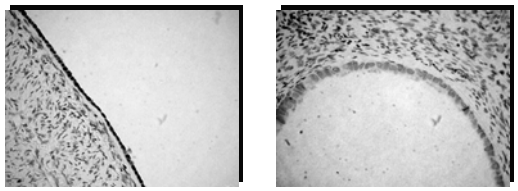
Validation of Genes Differentially Expressed in Cyst and Tumor Vs. Surface Epithelium

- 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)

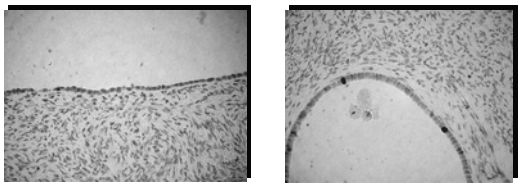
FOS expression in ovarian surface and cystic epithelium



IHC	Surface Epithelium	Cyst Epithelium
Negative or Weak	12 (26%)	52 (93%)
Strong	35 (74%)	4 (7%)
Total	47 (100%)	56 (100%)

P = 0.001

TOP2A expression in ovarian surface and cystic epithelium



IHC	Surface Epithelium	Cyst Epithelium
Negative	43 (91%)	35 (65%)
Positive	4 (9%)	19 (35%)
Total	47 (100%)	54 (100%)

P = 0.002

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

Expression Analysis Systematic Explorer (EASE)

- http://david.niaid.nih.gov/david/ease.htm
- 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

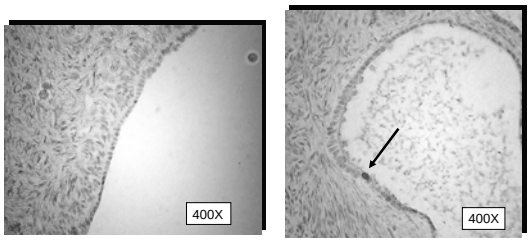
- Down-regulated: signal transduction activity (*n* = 31); *P* = 0.004
- Up-regulated: mitotic cell cycle (*n* = 12); *P* = 0.03
- Up-regulated: microtubule organization and biogenesis (*n* = 6); *P* = 0.007

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



Surface epithelium Cystic inclusion

Cell Proliferation

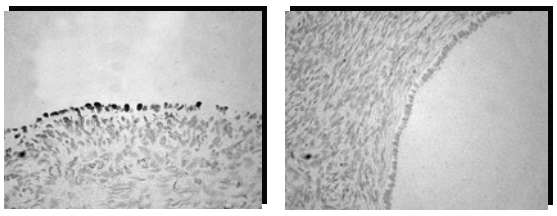
Cell type	# samples	# nuclei	# positive nuclei
Ovaries	36	39,832	220 (0.55%)
Surface	28	19,216	64 (0.33%)
Cystic	32*	20,616	156 (0.76%)

*159 inclusion cysts *P* = 0.0001

Rate of apoptosis in cyst vs. surface?

(TUNEL assay)

Assessment of Apoptosis by TUNEL



Surface epithelium Cystic inclusion

Apoptosis

Cell type	# samples	# nuclei	# positive nuclei
Ovaries	12	10,270	398 (3.9%)
Surface	9	2,197	274 (12%)
Cystic	10*	8,073	124 (1.5%)

*89 inclusion cysts

P = 0.0001

"Cell Proliferation Index"

Cell type	Ki-67	Apoptosis	Ratio*
Surface	0.33%	12%	0.028
Cystic	0.76%	1.5%	0.51
Cyst to surface			18

Aneuploidy in pathologically normal ovarian cystic inclusions?

DNA Content in Archival Ovarian Tissues

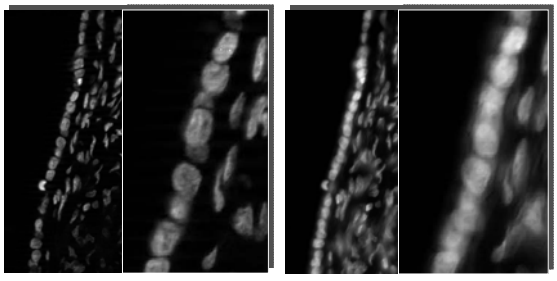
- FFPE normal ovaries cut into 10 µM sections (to avoid sectioning through nuclei
- Aneuploid MCF7 and SKBR3 breast cancer cell lines used as positive controls
- Slides deparaffinized and stained with propidium iodide
- Slides imaged using Zeiss Axioplan 2 microscope

DNA Content in Archival Ovarian Tissues

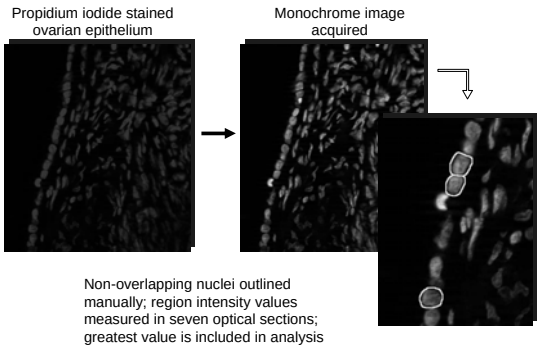
- Images captured at 400X with AxioCam MR monochrome digital camera providing a resolution of 1300 x 1030 pixels using the ApoTome (enhanced) confocal imaging system
- Using AxioVision image acquisition software, 7 optical sections are captured per high-power field at 0.85 µM intervals
- Monochrome images 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

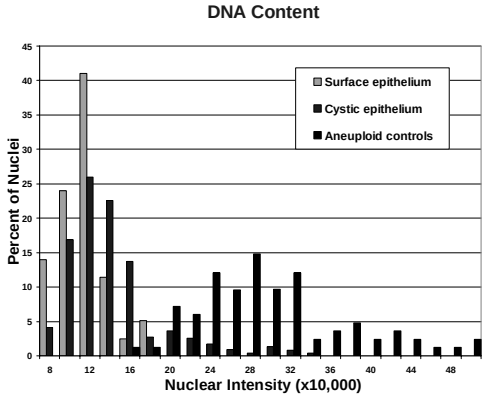
- ApoTome image (left) compared to conventional wide-field confocal image (right). ApoTome image is sharper and better focused.



Nuclear Image Acquisition and Quantitation



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



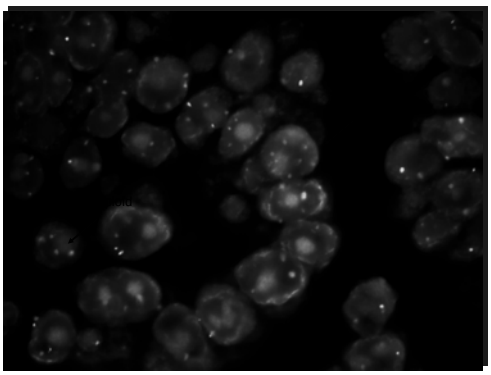
DNA Content

Cell type	# nuclei	Mean intensity/nucleus
Surface	78	127,215
Cystic	217	151,831
Aneuplod control	82	309,877
Total	377	181,114
		P = 0.001

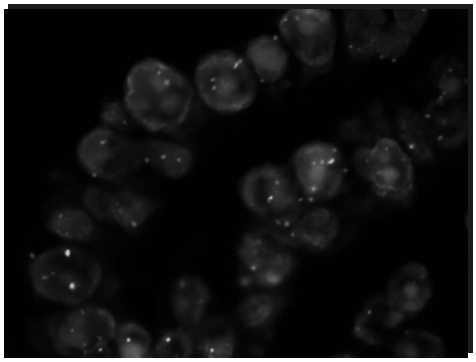
Karyotypic aneuploidy?

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

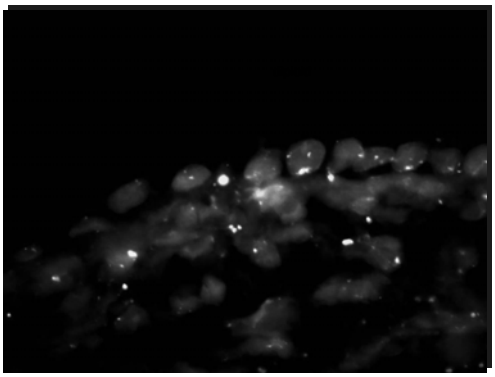
MCF7 cells (aneuploid)



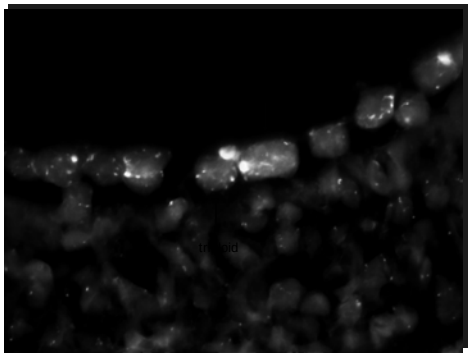
MCF7 cells (aneuploid)



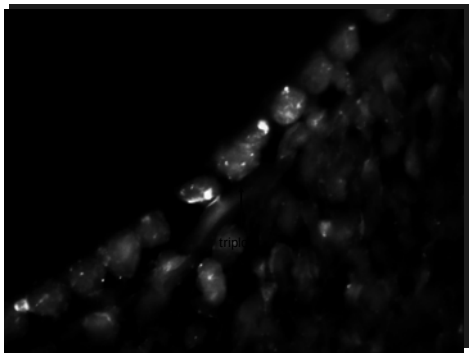
Surface epithelium



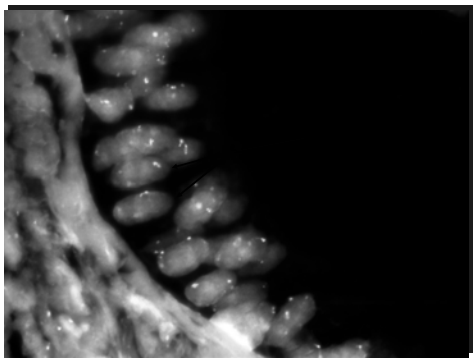
Inclusion cyst



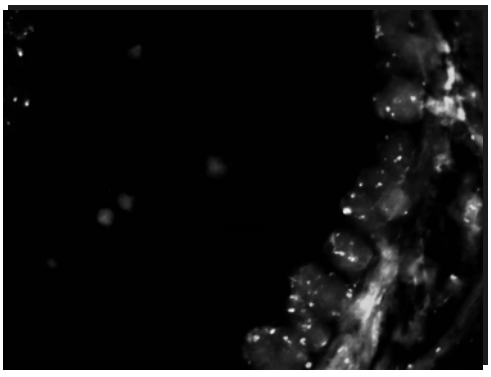
Inclusion cyst



Inclusion cyst



Inclusion cyst



Aneuploidy

Ploidy	Surface	Cystic
(Total cells)	410	880
Diploid	408 (99.5%)	799 (90.8%)
Aneuploid	2 (0.5%)	81 (9.2%)

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 Soslow, 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

National Cancer Institute

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

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

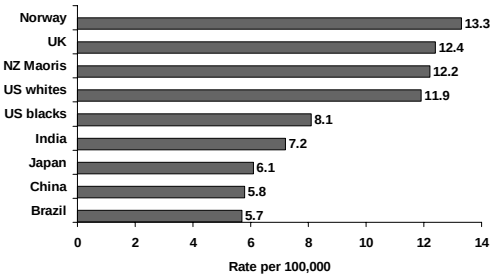
Overview of Ovarian Cancer Risk

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

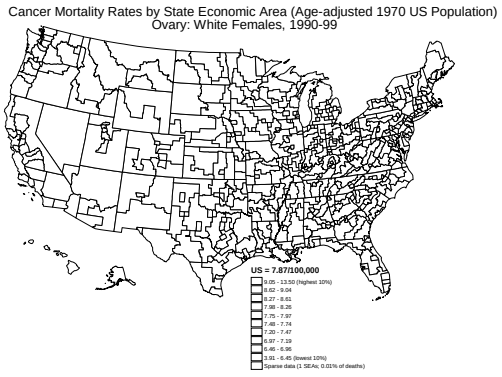
Age-Specific Incidence Rates

SEER, 1973-2002

Incidence Varies Between Countries



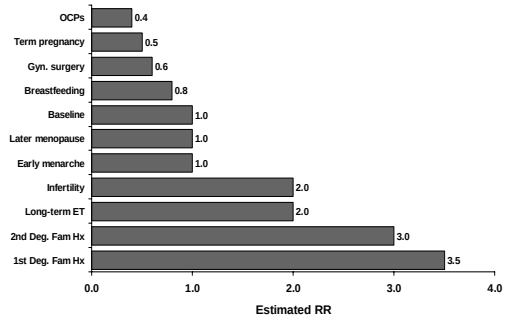
Parkin DM et al., Cancer Incidence in Five Continents 1997



Summary Lifetime Risks *SEER, 1999-2001*

	US White	US Black
Probability of developing	1.4 %	0.8 %
Birth to 39	0.07 %	0.06 %
40-59	0.4 %	0.2 %
60-84	1.1 %	0.7 %
Probability of dying from	1.0 %	0.6 %

Risk and Protective Factors



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

Sterling D, Evans DGR, Pichert G, Shenton A, Kirk EN, Rimmer S, Steel CM, Lawson S, Busby-Earle RMC, Walker J, Lalloo FI, Eccles DM, Lucassen AM, Porteous ME. J of Clin Oncol. 2005;23(24):5588-96.

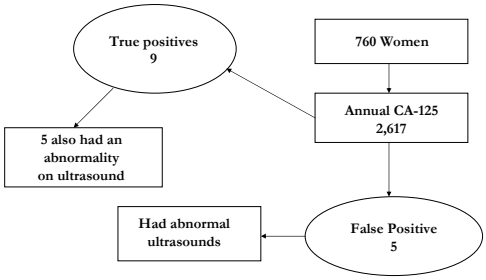
Performance of TVU and CA-125

	Sensitivity		PPV	
Parameter	**%**	**95% CI**	**%**	**95% CI**
TVU	46.2	19.1 – 73.3	17.1	4.7 – 29.6
Serum CA-125	81.8	59.0 - 100	63.4	39.2 – 89.4

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

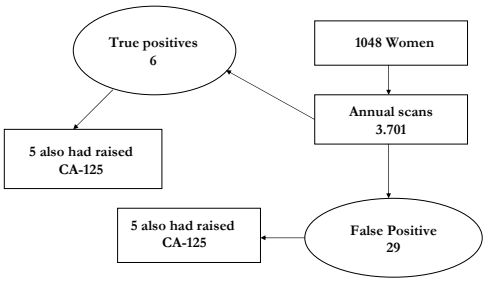
Sterling et al. 2005

CA-125 Outcome Measurements



Sterling et al. 2005

Ultrasound Outcome Measures



Sterling et al. 2005

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

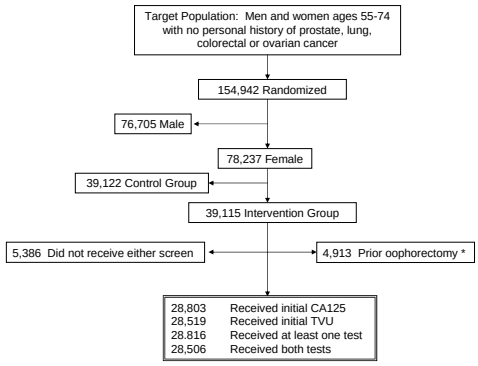
Ian Jacobs. J of Clin Oncol. 2005;23(24):5443-5.

Ovarian Cancer Screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial:

Findings from the Initial Screen of a Randomized Trial

Buys SS, Partridge E, Greene MH, Prorok P, Reding D, Riley T, Hartge P, Fagerstrom R, Ragard L, Izmirlian G, Fouad M, Johnson C, Gohagan J. Am J Ob Gyn (In Press).

Flow of participants into the PLCO Trial



* Ineligible for screening

Characteristics of participants in the intervention arm

	Number	%
AGE GROUP		
55 - 59	13459	34.4
60 - 64	11774	30.1
65 - 69	8588	22.0
70 - 74	5294	13.5
RACE		
White	33828	86.5
Black	2170	5.5
Hispanic	605	1.5
Asian	1259	3.2
Other	283	0.7
Missing Response	970	2.5

Characteristics of participants in the intervention arm (continued)

EDUCATION LEVEL	Number	%
< High school	2520	6.4
12yrs/Completed High School	15299	39.1
Some College	8911	22.8
College Graduate	5828	14.9
Postgraduate	5537	14.2
Missing Response	1020	2.6
HAD PRIOR PELVIC SURGERY		
Bilateral Oophorectomy	64	0.2
Hysterectomy	9065	23.2
Bilateral Oophorectomy & Hysterectomy	4789	12.2
Neither	24114	61.6
Missing Response	1083	2.8
EVER TAKEN ORAL CONTRACEPTIVES		
No	17622	45.1
Yes	20465	52.3
Missing Response	1028	2.6

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

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

Epidemiology: Risk Factors

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.

North Carolina Ovarian Cancer Study

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

<u>Study Subjects:</u> 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

No. of ovulatory cycles	No. of p53-positive cases	No. of control subjects	OR*	95% CI
≤ 234	4	840	1.0	Referent
235-375	29	1222	4.3	1.4-13.0
376-533	67	1159	9.1	2.7-30.9

*Adjusted by age, age^2, menopausal status, and nulliparity.

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

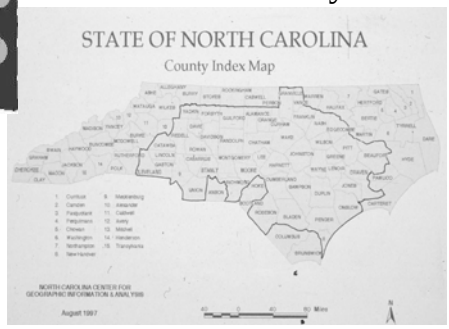
No. of ovulatory cycles	No. of p53-negative cases	No. of control subject	OR*	95% CI
≤ 234	23	840	1.0	Referent
235-375	19	1222	0.6	0.3-1.4.8
376-533	45	1159	1.3	0.5-3.2

*Adjusted by age, age^2, menopausal status, and nulliparity.

<u>STUDY DESIGN</u>

- 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)

SUBJECT TYPE	ENROLLED
Epithelial Ovarian Cancer Patients	786
Peritoneal Cancer Patients	62
Control Subjects	831
Total	1679

Objective

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

Case-control comparison of lifetime ovulatory cycles:
Cyclin E-postive vs. control subjects

No. of ovulatory cycles	No. of Cyclin E positive cases	No. of control subject	OR*	95% CI
< 265	20	188	1.0	Referent
265-390	50	185	2.3	1.2-4.2
> 390	80	175	3.8	2.0-7.3

*Adjusted by age, age^2, race, menopausal status

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

No. of ovulatory cycles	No. of Cyclin E negative cases	No. of control subject	OR*	95% CI
≤ 264	35	188	1.0	Referent
265-390	52	185	1.5	0.9-2.5
> 390	37	175	1.3	0.7-2.4

*Adjusted by age, age^2, race, menopausal status

Case-control comparison of Oral Contraceptive Use (OC):
Cyclin E-positive vs. control subjects

Years of OC Use	No. of Cyclin E Positive cases	No. of control subject	OR*	95% CI
0	91	219	1.0	Referent
≤ 5	75	283	0.6	0.4-1.0
> 5	32	215	0.4	0.2-0.6

*Adjusted by age, age^2, race, menopausal status

Case-control comparison of Oral Contraceptive Use (OC): Cyclin E-negative vs. control subjects

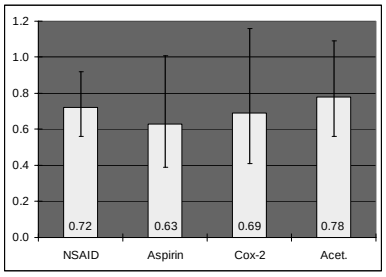
Years of OC Use	No. of Cyclin E negative cases	No. of control subject	OR*	95% CI
0	55	219	1.0	Referent
≤ 5	65	283	0.9	0.6-1.3
> 5	52	215	0.9	0.6-1.5

*Adjusted by age, age^2, 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).

Odds Ratios and 95% CIs for association between types of analgesics and ovarian cancer



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

	GG	AG	AA	AG/AA (%)	OR*	(95% CI)
Controls	445	58	1	59 (11.7)	1.00	Reference
Serous	244	26	0	26 (9.6)	0.81	(0.50 - 1.32)
Mucinous	44	5	0	5 (10.2)	0.80	(0.30 - 2.14)
Endometrioid	53	3	0	3 (5.4)	0.43	(0.13 - 1.40)
Clear cell	23	0	0	0 (0.0)	-	- -
Endometrioid/clear cell	76	3	0	3 (3.8)	0.30	(0.09 - 0.97)
Mixed	1	0	0	0 (0.0)	-	- -
Other	35	3	1	4 (10.3)	0.86	(0.29 - 2.51)

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

Conclusion

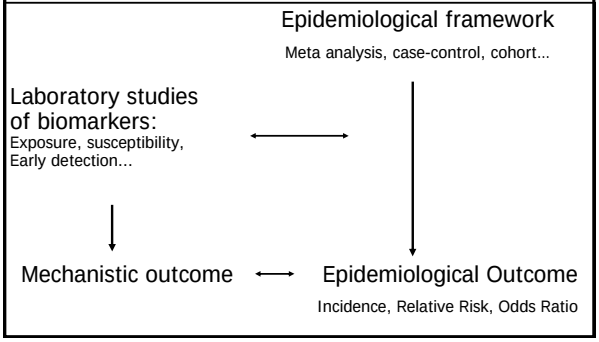
- **The +331G/A progesterone receptor polymorphism may modify the molecular epidemiology pathway that encompasses the development of endometrioid/clear cell ovarian cancer possibly via subsequent transformation from endometriosis.**

New Directions

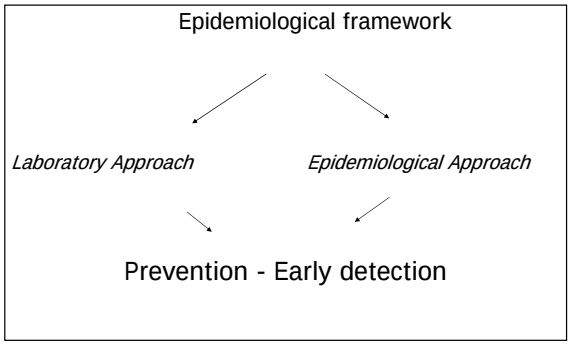
SUMMARY

SUMMARY

GENERAL MODEL FOR MOLECULAR EPIDEMIOLOGICAL STUDIES



The Approach to Ovarian Cancer



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

Adami HO et al, Lancet 1994; 344: 1250-4

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

Two Large Cohorts

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

Study Aims

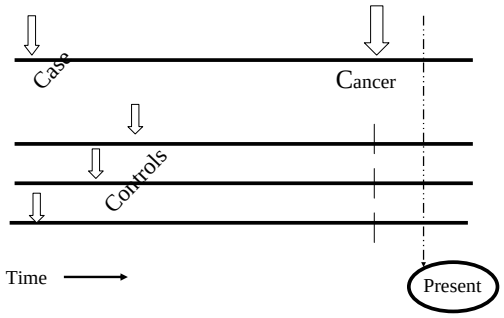
- Maternal risk of epithelial ovarian cancer associated with circulating
  - Progesterone (P)
  - 17α-OH progesterone (OHP)

  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



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

Site	Borderline	Invasive	Total
Finland	687	957	1644
Sweden	56	127	183
Total	**743**	**1084**	**1827**

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
FOX CHASE CANCER CENTER

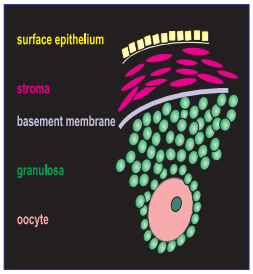
Ovarian cancer

- 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)**

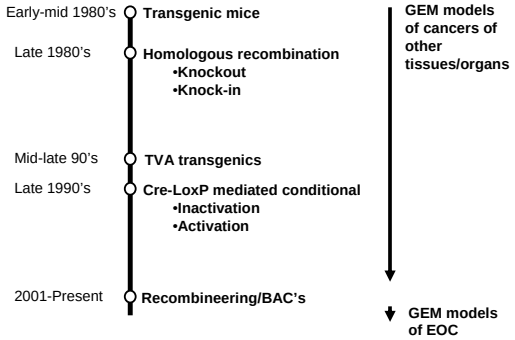
Animal models of epithelial ovarian cancer (EOC)

- 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



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

Promoter identification strategies

- Theoretical: (e.g., FSHR and MISIIR)
- Literature review: (e.g., Mesothelin)
- Experimental: (e.g., CK19)

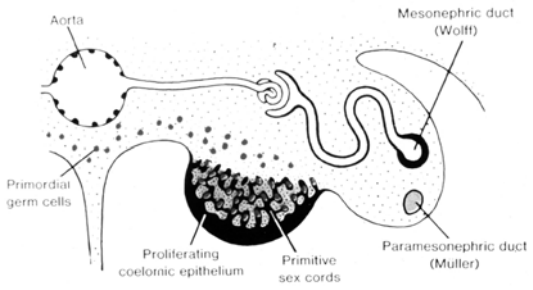
Candidate validation

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

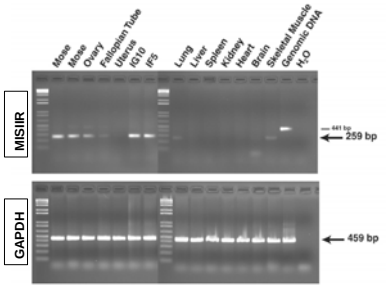
Müllerian inhibitory substance (MIS) signaling

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.



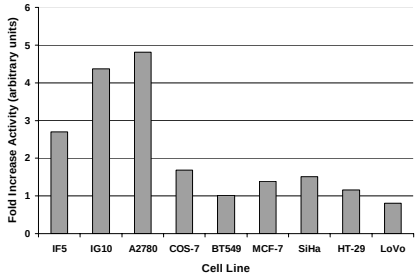
RT-PCR analysis of MISIIR expression in mouse tissues



Mouse MISIIR promoter

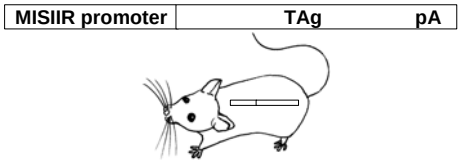
- PCR cloned based on homology to published rat MISIIR promoter sequence and mouse genomic DNA sequences
  - 1204 bp fragment that shares >95% sequence homology to rat promoter

In Vitro transcriptional activity of the mouse MISIIR promoter

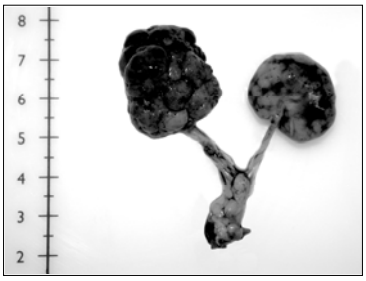


MISIIR-TAg construct

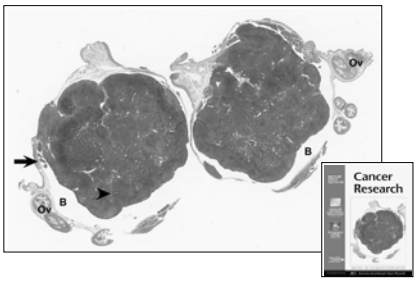
- Murine 5' upstream regulatory region of the Mullerian inhibiting substance type II receptor (MISIIR) gene PCR fused to the early region of SV40 including the large and small T antigen genes (provided by D. Hanahan)



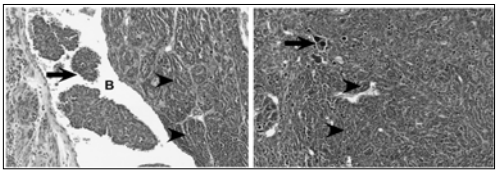
Bilateral ovarian tumors in a *MISIIR-TAg* transgenic mouse



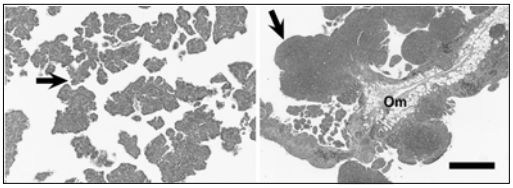
ScanScope view of ovaries substituted by tumor cells



Tubular and papillary structures and floating buds in intrabursal space



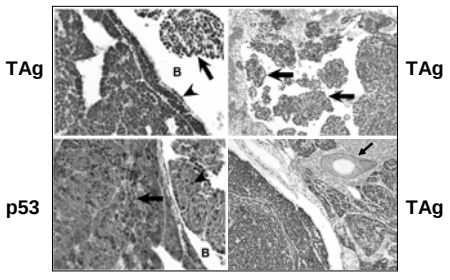
Papillary structures apparent in ascites and invasion of omentum



Histological Features

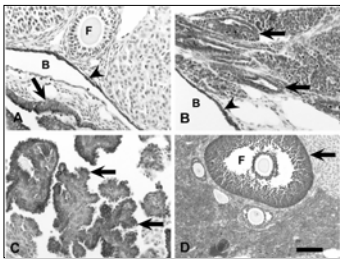
- Poorly differentiated carcinomas with cysts and papillary structures present at the surface of the ovary
- Intraperitoneal dissemination with invasion of omentum, implants in peritoneal organs and formation of ascites

Tumors express TAg and p53



Tumors express markers of epithelial cell differentiation

CK8



CK8

CK8

α–inhibin

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?

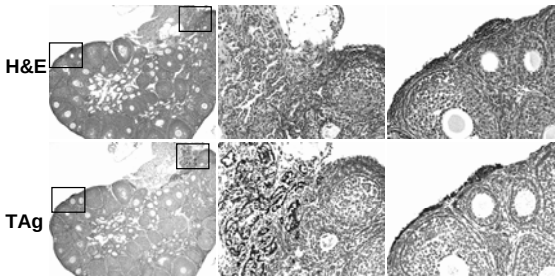
- Most female mice develop ovarian tumors prior to breeding
- ~28% of males develop Sertoli cell tumors
- Some female offspring of affected males develop ovarian tumors
- Targeting reproductive organs for tumorigenesis = poor breeding

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?

Four week-old mice:



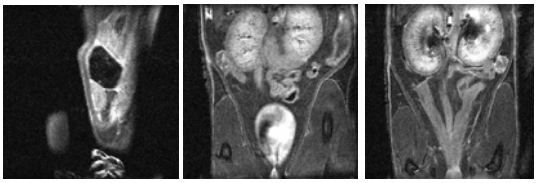
Therapeutics

- 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

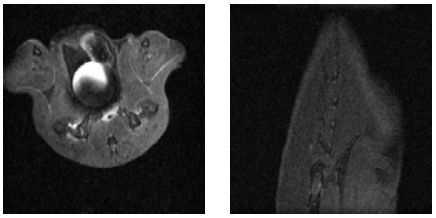
Magnetic Resonance Imaging (MRI) of mice

- Images acquired with a 2-D spin-echo pulse sequence, T_R=1200 msec, T_E=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

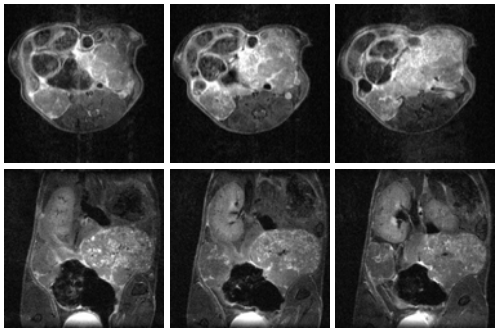
Normal mouse



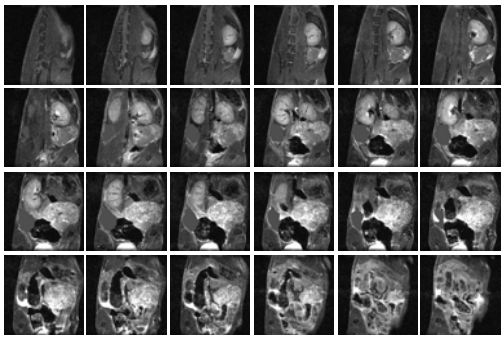
Tg*MISIIR-TAg* Mouse



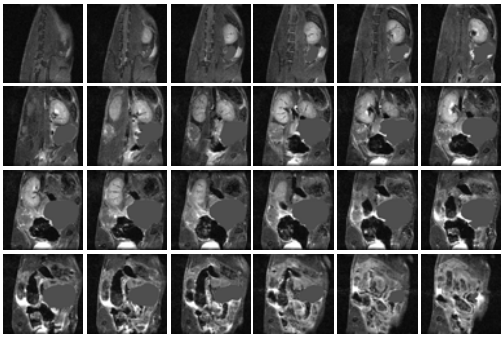
Tg*MISIIR-TAg* Mouse



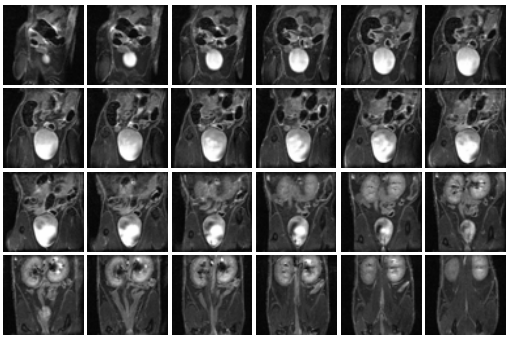
Right Ovarian Tumor



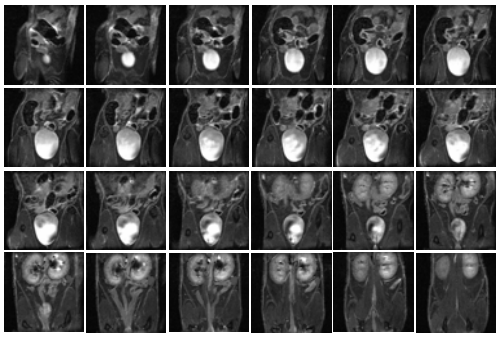
Left Ovarian Tumor



Normal Right Ovary



Normal Left Ovary

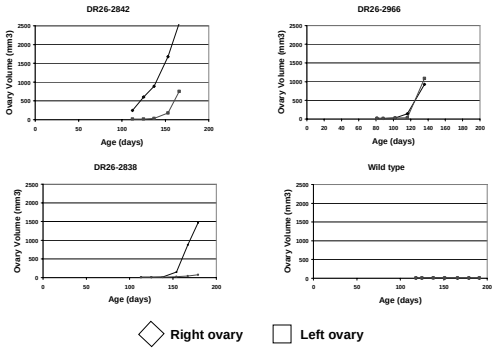


Volume Measurement

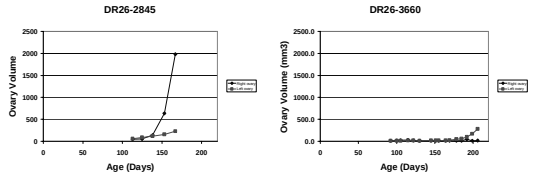
	Dimension	Size (mm)
X	256 pixels	0.1
Y	256 pixels	0.1
Z	32 slices	0.5

Formula: (0.1 mm)(0.1 mm)(0.5 mm)(nROI)

Tumor volumes



Cisplatin/Taxol treatment



Untreated **Treated**

Similarities between Tg*MISIIR-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 Tg*MISIIR-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

Fox Chase Cancer Center
Skye Harris
Christine Williams
Kim Baxter-Jones
Tiffany Brake, Ph.D.
Rudi Bao, M.D.
Xiang Hua
Harvey Hensley, Ph.D.
Tom Hamilton, Ph.D.
Cornell University
Alex Nikitin, M.D., Ph.D.
Ottawa Regional Cancer Center
Barbara Vanderhyden, Ph.D.
Cornell Medical School
Lora Hedrick-Ellenson, M.D.
National Cancer Institute
Mike Birrer, M.D., Ph.D.
Virna Leaner, Ph.D.
UCSF
Joe Gray, Ph.D.
Graeme Hodgson, Ph.D.
UPENN
Ron Wolf, M.D., PhD.

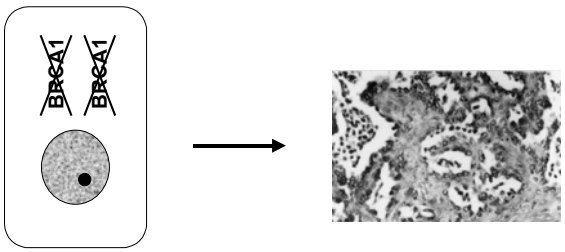
- NCI- Mouse Models of Human Cancer Consortium (MMHCC)
- U.S. Department of Defense
- Ovarian Cancer Research Fund
- NCI – Ovarian Cancer SPORE

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

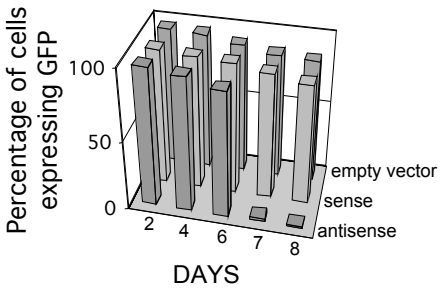
MECHANISM OF OVARIAN CANCER PREDISPOSITION IN BRCA1 MUTATION CARRIERS

Tumor suppressor hypothesis:



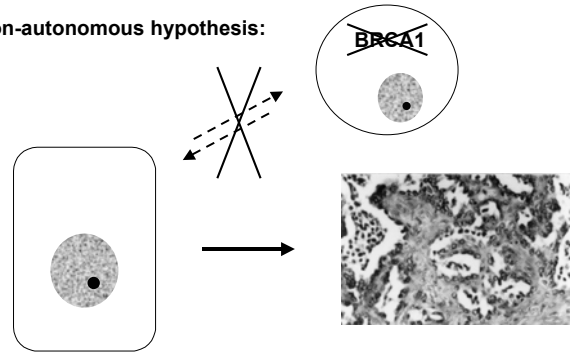
Cell of origin — Ovarian carcinoma

SELECTIVE DISADVANTAGE OF REDUCED *BRCA1* EXPRESSION



MECHANISM OF OVARIAN CANCER PREDISPOSITION IN BRCA1 MUTATION CARRIERS

Cell non-autonomous hypothesis:

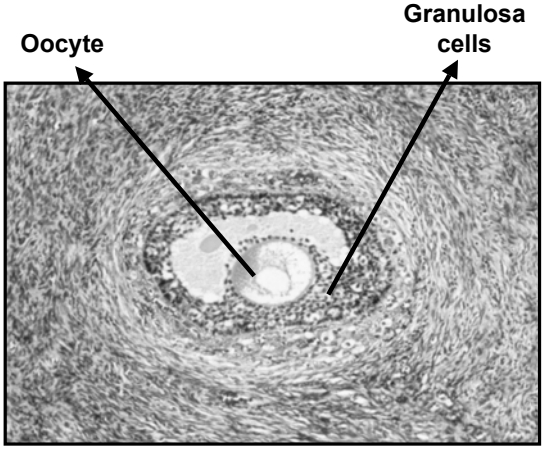


Cell of origin — Ovarian carcinoma

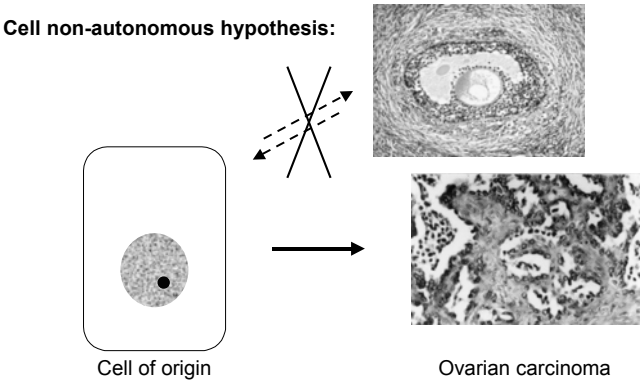
INFLUENCE OF PARITY AND ORAL CONTRACEPTIVE USE ON OVARIAN CANCER RISK

Risk Factors		Relative Risk
Parity	0	1.00
	1	0.61
	2	0.43
	3	0.40
	4+	0.31
Total Oral Contraceptive Use (yr)	0	1.0
	<1	0.9
	1-3	0.8
	3-5	0.5
	5+	0.4

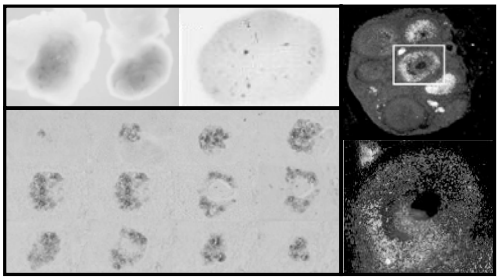
SECONDARY FOLLICLE



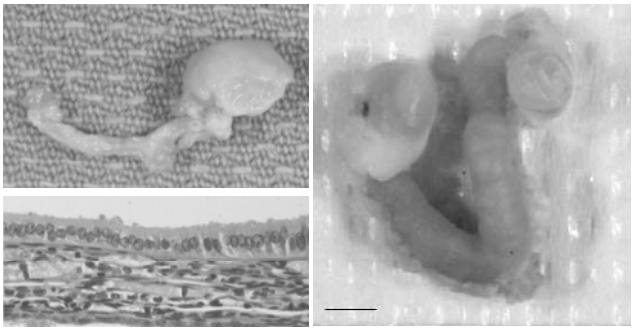
MECHANISM OF OVARIAN CANCER PREDISPOSITION IN BRCA1 MUTATION CARRIERS



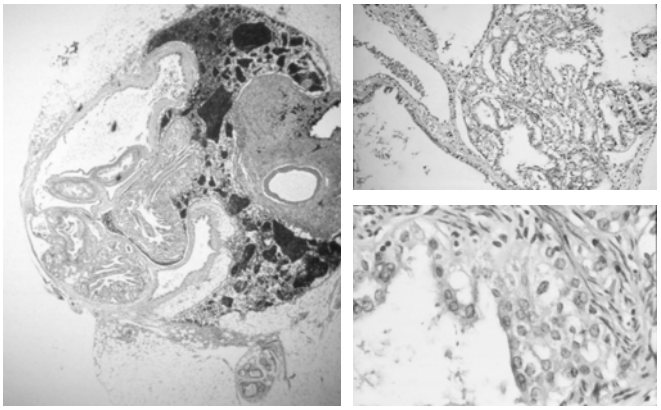
DISTRIBUTION OF PROMOTER ACTIVITY IN R26R MICE



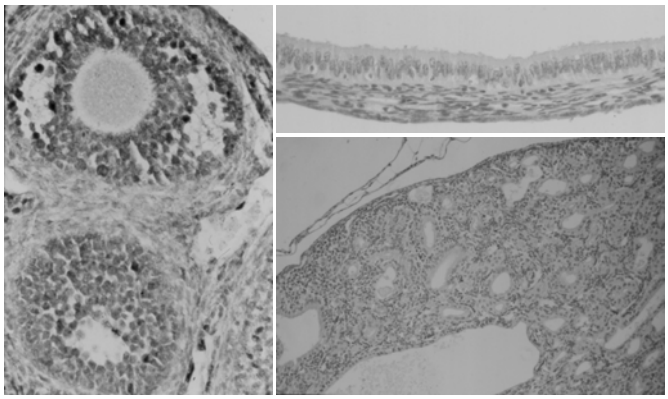
MUTANT MICE DEVELOP OVARIAN CYSTADENOMAS



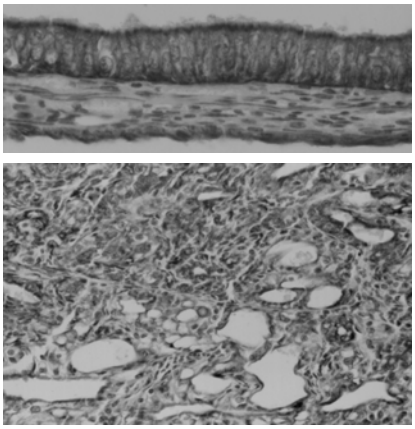
INVASIVE CARCINOMA IN A P53/BRCA1 DOUBLE MUTANT



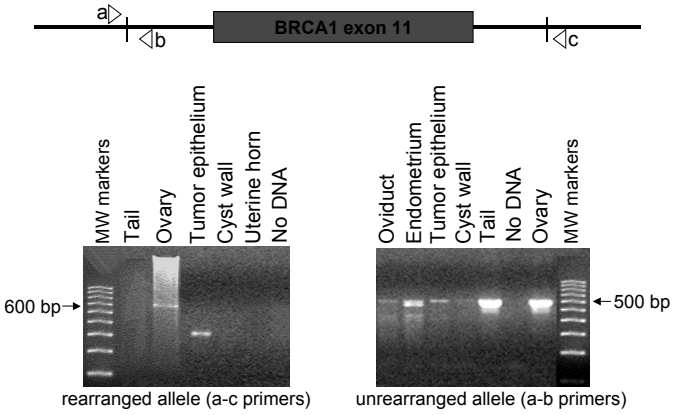
IMMUNOHISTOCHEMICAL STAINING FOR MULLERIAN INHIBITING SUBSTANCE



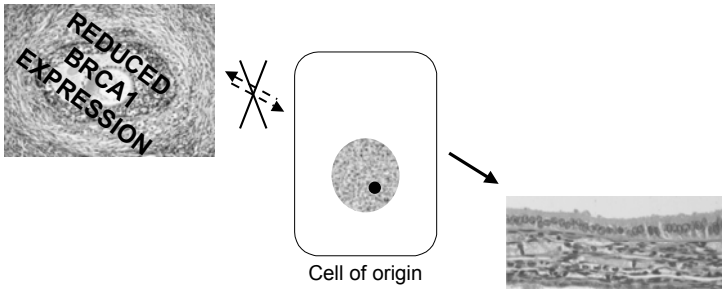
IMMUNOHISTOCHEMICAL STAINING FOR NON-SQUAMOUS KERATINS



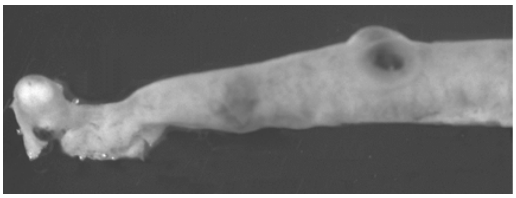
TISSUE DISTRIBUTION OF MUTANT ALLELES



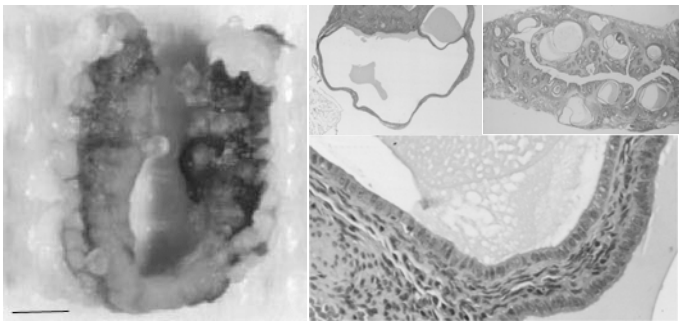
MECHANISM OF OVARIAN CANCER PREDISPOSITION IN BRCA1 MUTATION CARRIERS



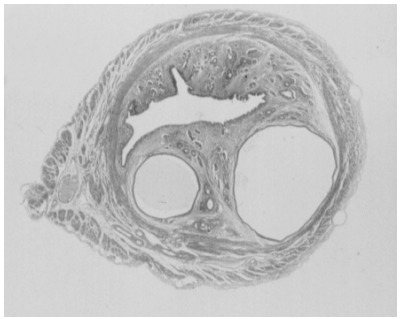
PERI-TUBAL CYST IN BRCA1 KNOCK-OUT MICE



MUTANT MICE DEVELOP EPITHELIAL CYSTS ALONG THE ENTIRE MULLERIAN TRACT



UTERINE CYST



Gynecologic Oncology **72,** 437–442 (1999)
Article ID gyno.1998.5275, available online at http://www.idealibrary.com on IDEAL®

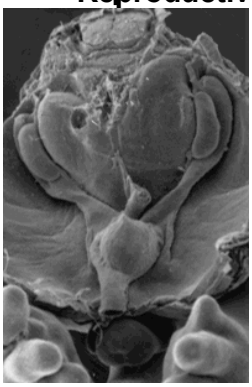
COMMENTARY

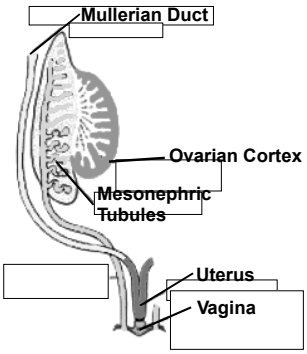
The Cell of Origin of Ovarian Epithelial Tumors and the Ovarian Surface Epithelium Dogma: Does the Emperor Have No Clothes?

Louis Dubeau

USC/Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, California 90033

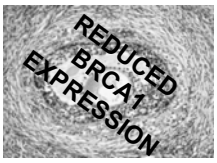
Embryological Development of the Female Reproductive System (9 weeks)





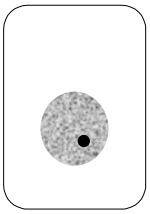
http://wwwmed.unc.edu/embryo_images/unit-welcome/welcome_htms/contents.htm

CELL NON-AUTONOMOUS MECHANISM OF PREDISPOSITION TO CANCERS OF THE REPRODUCTIVE TRACT IN BRCA1 MUTATION CARRIERS



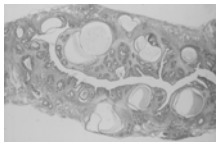
Ovarian granulosa cells

?



Mullerian epithelium

Ovarian cysts



Uterine cysts

POSSIBLE MECHANISMS FOR OVARIAN TUMOR PREDISPOSITION BASED ON A CELL NON-AUTONOMOUS SCENARIO

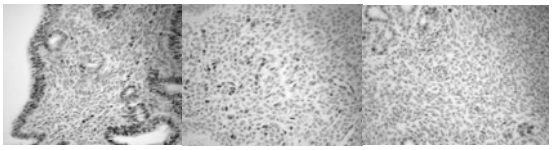
- Direct mechanism
  - An effector secreted by granulosa cells is regulated by Brca1
- Indirect mechanism
  - Brca1 inactivation results in alterations in the ovulatory cycle, which in turn influences ovarian epithelial tumor development

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

Age: 3-4 months

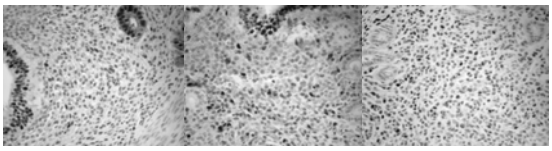
Estrus cycle stage: early diestrus

Wild type
36.1 +/- 24.8%



P = .025

Mutant
67.7 +/- 6.7%



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

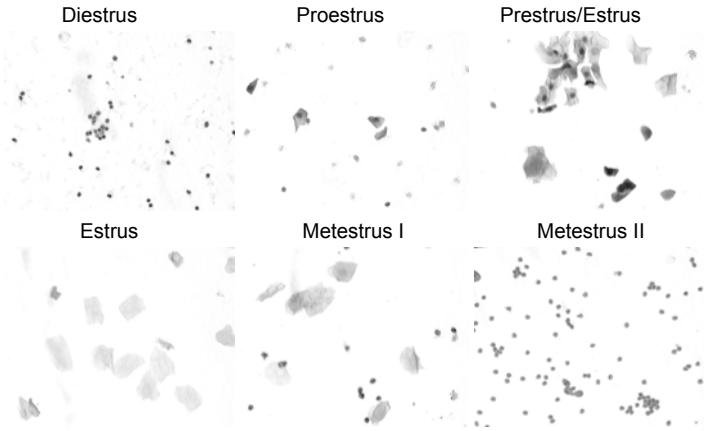
Yanfen Hu,*, Sagar Ghosh, Asma Amleh, Wei Yue,[1] Yunzhe Lu,[3] Adam Katz,[2] Rong Li *

Department of Biochemistry and Molecular Genetics
[1]Department of Medicine and Division of Endocrinology
[2]Department of Plastic and Reconstructive Surgery
School of Medicine
University of Virginia
Charlottesville, VA 22908

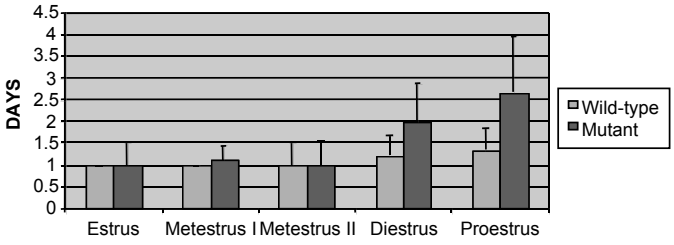
[3]State Key Laboratory of Genetic Engineering
Institute of Genetics
School of Life Science
Fudan University
Shanghai 200433, China

Oncogene, in press

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



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**

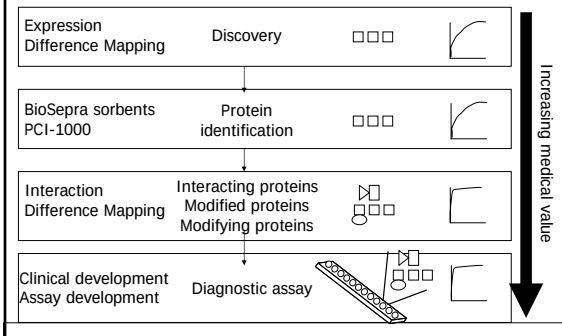
Proteomics vs Genomics

- **Proteins actually do the work of the cell**
  - DNA/RNA analysis cannot predict the amount of a gene product made (if 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)**

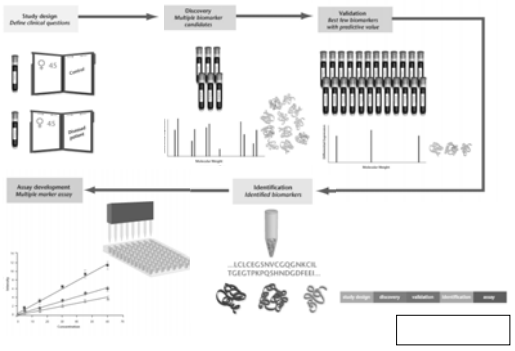
Protein biomarkers

- **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

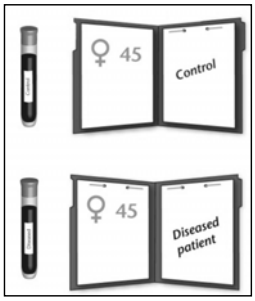


Pattern Track™ workflow



study design

Study design



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

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**

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 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

The clinical question

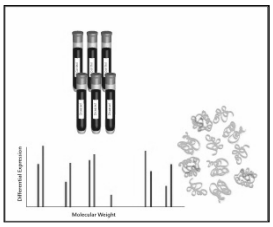
- **Clear-cut clinical question**
  - Unmet clinical need
  - The marker will affect patient management and patient outcome
- **Know the desired results**
- **Controls just as important as disease samples**

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

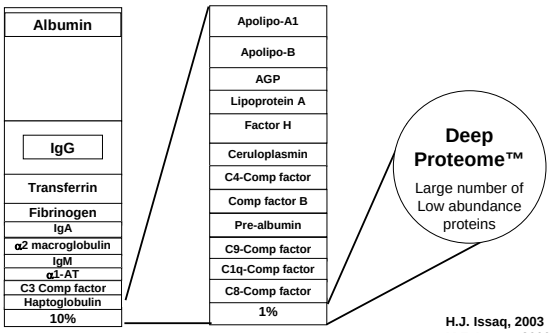
Discovery phase



Pilot study to discover multiple biomarker candidates requires:

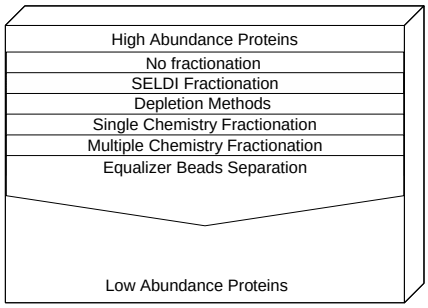
- **High sensitivity**
- **Increased resolving power**
- **Broad dynamic range**

Dynamic range of the plasma proteome



H.J. Issaq, 2003
M.F. Lopez, 2000

Use pre-fractionation methods to access the Deep Proteome™

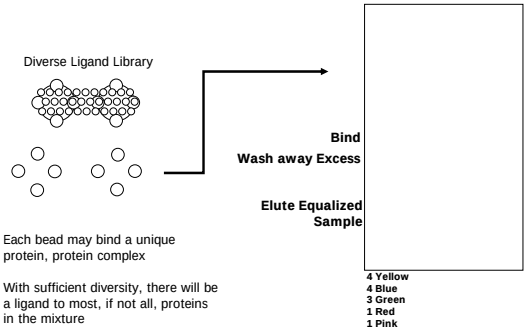


Equalizer Beads: Dynamic Range Compression An Addition to the Proteomic Toolbox

Decrease the dynamic range - dilute abundant and concentrate trace proteins

- Based on a combinatorial ligand library synthesized on beads
  - *Millions of copies of one unique ligand per bead*
  - *10^9 to $>10^{12}$ unique beads*
- Highly specific affinity interactions between proteins and ligands ensure binding the greatest number and variety of proteins
- Controlled bead capacity allows maximum concentration of trace proteins with dilution of high abundance proteins

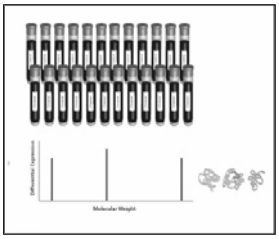
Principle of Protein Equalizer Beads



- Each bead may bind a unique protein, protein complex
- With sufficient diversity, there will be a ligand to most, if not all, proteins in the mixture

validation

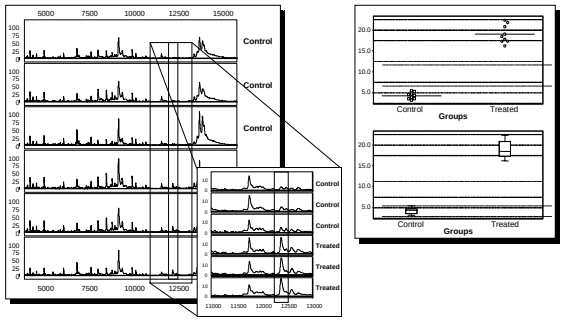
Validation phase



Selection of 'best few' biomarker candidates

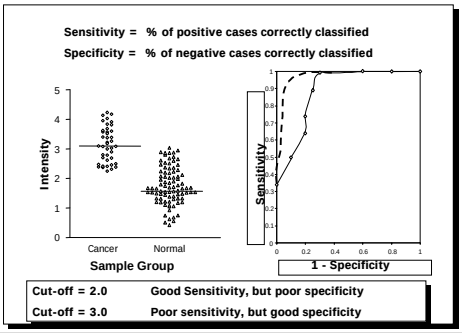
- **Advanced software tools**
- **High-throughput capabilities**
- **Reproducible validation tools**

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, heirarchal 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

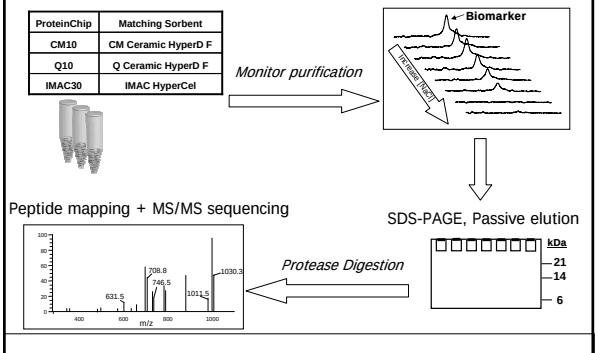
- **Divide discovery data into training and testing sets**
- **Feature selection: reduce from 1000s to <10 important variables**
- **Different methods of feature selection can lead to slightly different ranks of features but most important features will be common**
- **Further reduce number of features 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**

identification

Purification and identification phase

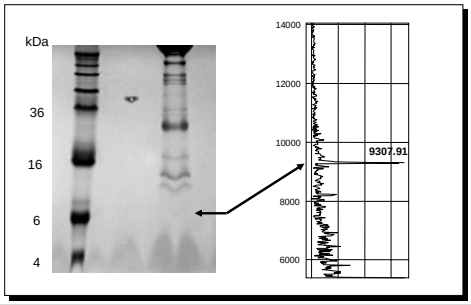
- **Purify selected biomarkers**
- **Use matching chromatographic resins.**
- **Peptide mapping and MS/MS sequencing for identification.**

SELDI-Assisted Purification and ID



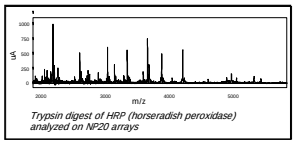
Biomarker Purification

9.3 kDa biomarker passively eluted from a 1D gel



Biomarker Identification

Peptide mapping and MS/MS sequencing

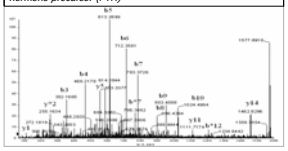


Trypsin digest of HRP (horseradish peroxidase) analyzed on NP20 arrays

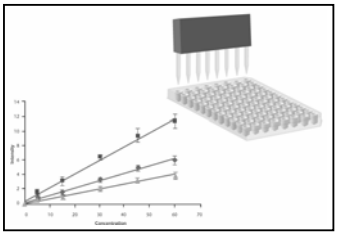
Peptide Mapping on the ProteinChip System, Series 4000

SELDI-MS/MS analysis on the ProteinChip Interface for ABI/Sciex QSTAR MS

MS/MS analysis of 1576 m/z peptide identifies Parathyroid hormone precursor (PTH)



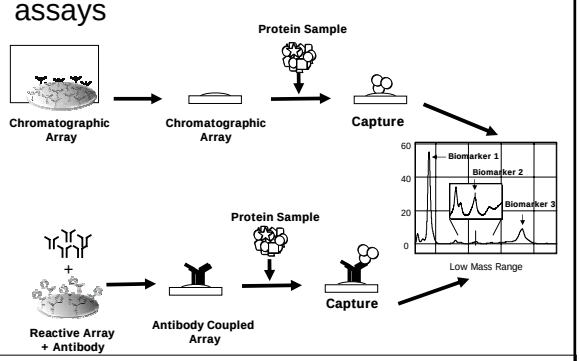
Assay Phase



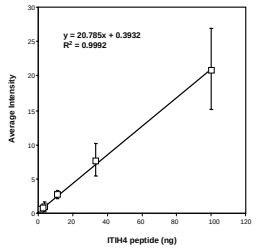
Develop multi-marker assays with:

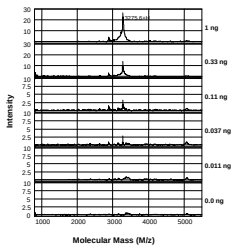
- **High predictive value**
- **Resolving power**
- **Quantitation capabilities**

Affinity and chromatographic based assays



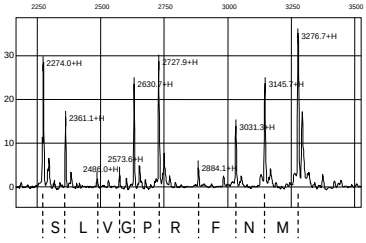
Standard Curve of ITIH4 Peptide





SELDI immunoassay reveals that ITIH4 is highly processed

. . .AGAAGSRMNFRPGVLSSRQLGLPGPPDVPDHAAYHPFRRLAILP...



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%

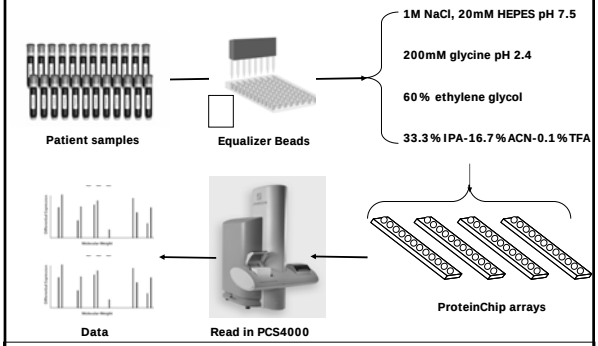
Precision of transthyretin peaks: 7-10 %

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

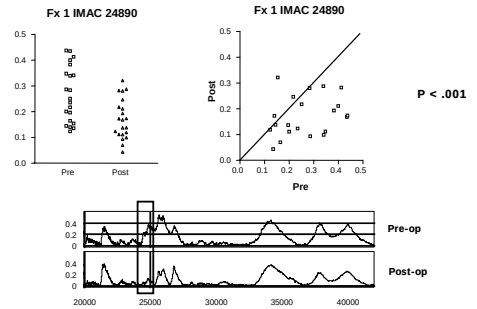
- **Spectra were calibrated and normalized to total ion current**
- **884 unique peaks across 16 conditions (4 fractions * 4 chip types)**
- **Peaks assessed for significance for**
  - Change in levels after surgery (using paired t-test comparing pre- and post-operative samples)
  - Predicting outcome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

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

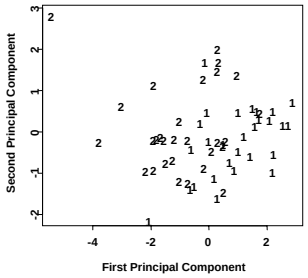
m/z	Fraction	Chip	Paired p value	AUC*
8350.95	2	IMAC30	0.0005	0.71358
24890.99	1	IMAC30	0.0006	0.72381
15900.05	1	IMAC30	0.0006	0.67619
6841.48	2	H50	0.0014	0.303704
19986.58	1	H50	0.0016	0.290123
8152.45	2	CM10	0.0019	0.736905
8144.70	2	IMAC30	0.0019	0.72716
15514.24	1	IMAC30	0.0019	0.667857
13567.82	1	Q10	0.0020	0.678322
165485.60	1	IMAC30	0.0029	0.319048

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

Peak down-regulated in response to surgery



Separation of pre- and post-treatment samples using PCA



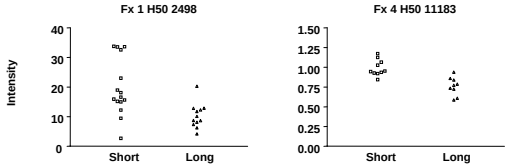
Peaks most strongly associated with outcome (pre-treatment samples)

m/z	Fraction	Chip	p value	AUC
11183.28	4	H50	0.0011	0.066667
2498.41	1	H50	0.0029	0.155556
23068.54	2	IMAC30	0.0034	0.155556
66956.90	2	IMAC30	0.0040	0.175
76483.84	4	CM10	0.0043	0.1
43124.27	2	CM10	0.0046	0.194872

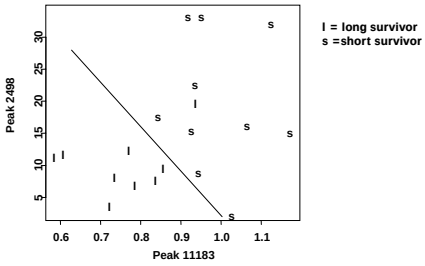
CA125 p value is not significant.

All peaks are higher in the short time to relapse group.

Scatter plots of two peaks



Separation of long and short survivors with pre-treatment samples



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**

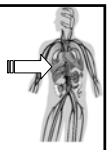
Application of Proteomic Technologies For Advances in Ovarian Cancer

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.

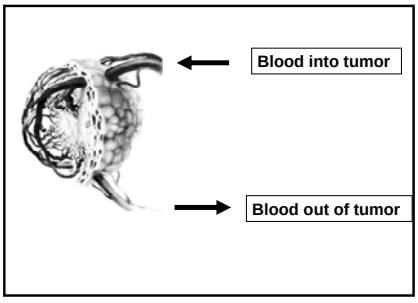
Elise C. Kohn, MD
Head, Molecular Signaling Section
Chief, Medical Ovarian Cancer Clinic
Center for Cancer Research
National Cancer Institute
Acting Director, Medical Education Program
National Institutes of Health

An Ideal Screening Test



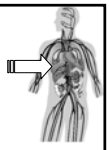
- **Sufficiently sensitive to detect early stage disease**
- **Specific enough to identify those without disease**
- **Easy to administer**
- **Effective intervention available**

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



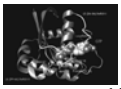
MOLECULAR HUNTING GROUNDS:

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**

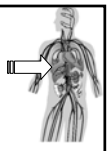
What Is Proteomics?

***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

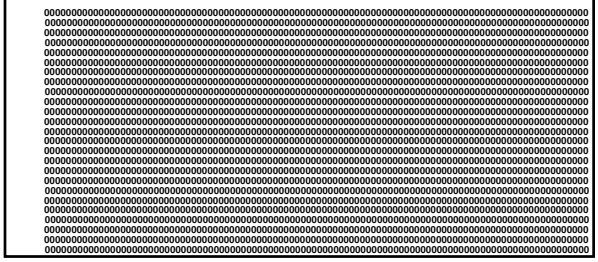


Specificity--
- **Heterogeneity of cancer**
- **Individuality of cancer**
- **Uniqueness of cancer from common processes**

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

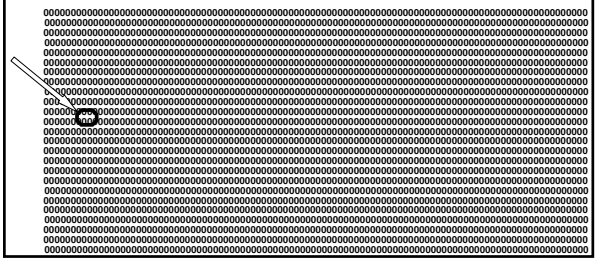
CHALLENGES IN TRANSLATING MARKERS TO CLINICAL USE:
Ovarian cancer affects 1:2500 post-menopausal women

Can you find the yellow 0?



CHALLENGES IN TRANSLATING MARKERS TO CLINICAL USE:
Ovarian cancer affects 1:2500 post-menopausal women

SPECIFICITY CRITICALLY IMPORTANT
98% SPECIFICITY GIVES A 2% PPV
(49/50 women to surgery unnecessarily to find 1 cancer)

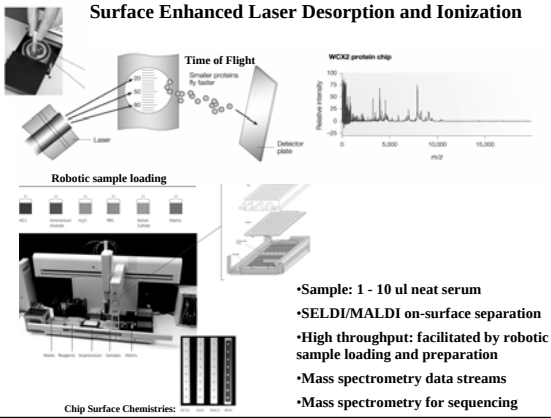


SERUM PROTEOMIC PATTERN DIAGNOSTICS

Serum protein signature diagnostics through mass spectrometry and bioinformatics.

QuickTime™ and a Photo - JPEG decompressor are needed to see this picture.

Surface Enhanced Laser Desorption and Ionization



- **Sample: 1 - 10 ul neat serum**
- **SELDI/MALDI on-surface separation**
- **High throughput: facilitated by robotic sample loading and preparation**
- **Mass spectrometry data streams**
- **Mass spectrometry for sequencing**

Ovarian Cancer

Stage	Description	Incidence	Survival
I	Confined to ovaries	20%	90%
II	Confined to pelvis	5%	65%
III	Spread IP or nodes	58%	45%
IV	Distant metastases	17%	<5%

Soon to open: GOG-220
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 c omplete 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, Duke, MDA, U-Wash, Cedars Sinai, Evanston and Northwestern Univ Hosp.

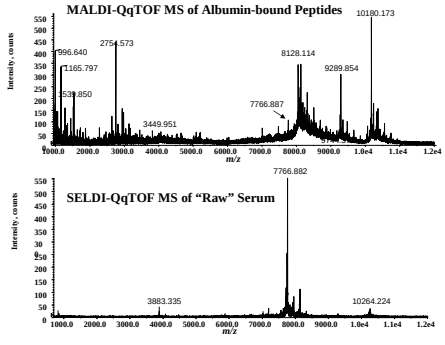
Referrals: NCI Clinical Studies Support Center 1-888-624-1837

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

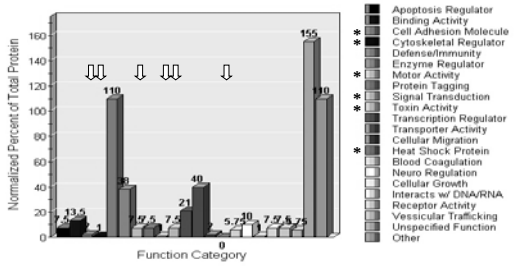
Sequencing from bound proteins yields reliable peptide identification



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

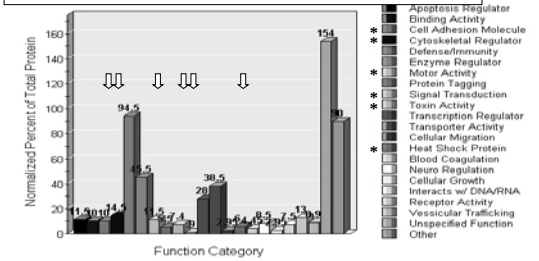
Categorization of Protein Function- Stage III OvCa

albumin-bound sequenced peptides from 20 pts; repeat verification



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**

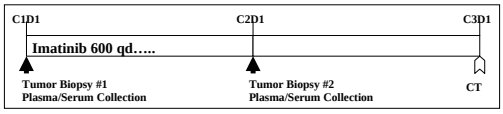
(1.5 million post-translational processing)

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

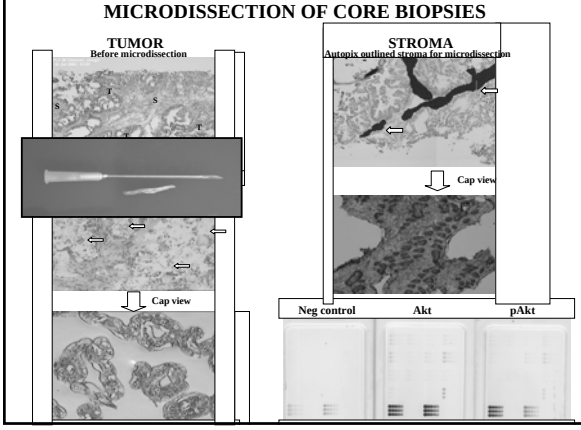
Clinical Objective: To determine clinical activity and toxicity profile of imatinib.

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

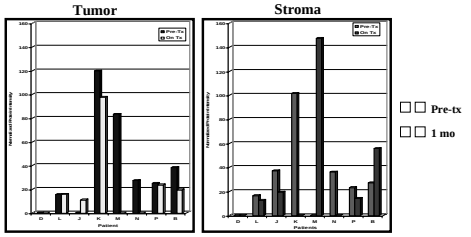
Eligibility Criteria:
1. Biopsiable recurrent epithelial ovarian cancer (biopsies required)
2. No more than 4 prior treatment regimens
3. Good end organ function



MICRODISSECTION OF CORE BIOPSIES



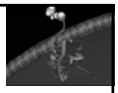
c-Kit Tyr 721 Phosphorylation is Reduced in Tumor and Stroma By Imatinib Therapy: *Proof of Target*



SCF stimulates p85 PI3K binding to Tyr-721 c-kit; PI3K → AKT and JNK to activate survival signals. pAKT may be a useful measure of imatinib activity.

Lysate microarrays is a method for studying a large range of signals across many patients.

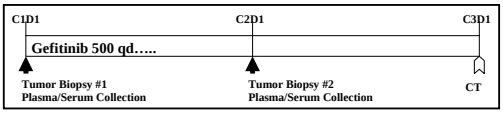
Phase II Trial with Proteomic Profiling of Gefitinib in Patients with Relapsed Ovarian or Cervical Cancer



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

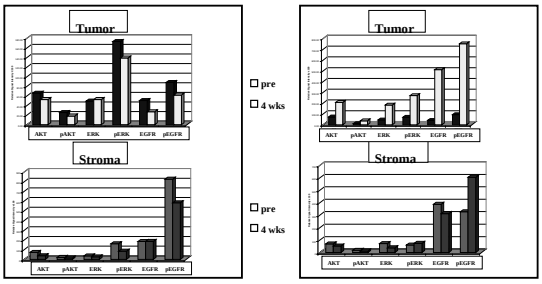
Secondary Objectives: To assess clinical activity and toxicity;
To correlate biochemical effects with outcome and toxicity;
To evaluate skin as a surrogate for tumor signal and clinical activity.

Eligibility Criteria: Relapsed epithelial ovarian cancer or cervix cancer
Biopsiable disease, Good end organ function
No limitation for prior number of therapies



* Imodium for diarrheal complications

Two example patients treated with gefitinib: Proteomic results



50 yo with Stage IV/gr 3 papillary serous ovarian carcinoma; 7 prior chemo regimens

Gefitinib 500 mg/d x 6 wks

Discontinued for gr 4 hemorrhage and splenic rupture. Patient anti-coagulated for aortic graft.

48 yo Stage IIIC/gr 3 papillary serous ovarian carcinoma; 10 prior regimens

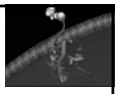
Gefitinib 500 mg/d x 8 wks

Disease progression at 8 wks

Adverse reactions- gr 2 fatigue

LIEL, PROC AACR '05

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 IHC
  - 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 Kotz
Lori Minasian
Gisele Sarosy
Debbie McNally
MOCRU Fellows and Nursing Staff

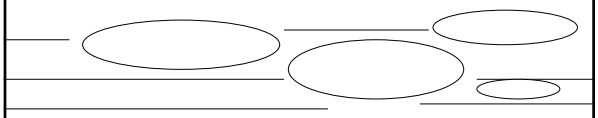
And the many other colleagues whose contributions could not be listed.

Ovarian Cancer Collaborators:

Gordon Mills	**Joe Gray**
David Fishman	**Ian Jacobs**
Nicole Urban	**Jan Vermorken**
Marty McIntosh	**Gus Rodriguez**
Steven Skates	**Monica Brown Jones**
George Coukos	
Ovarian SPORES	**GOG**

US/Italy Pharmacogenomics Consortium:
Sergio Pecorelli
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-RO1CA82562, NIH RO1 CA01015, 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 <u>carcinogenesis, invasion, metastasis</u>**

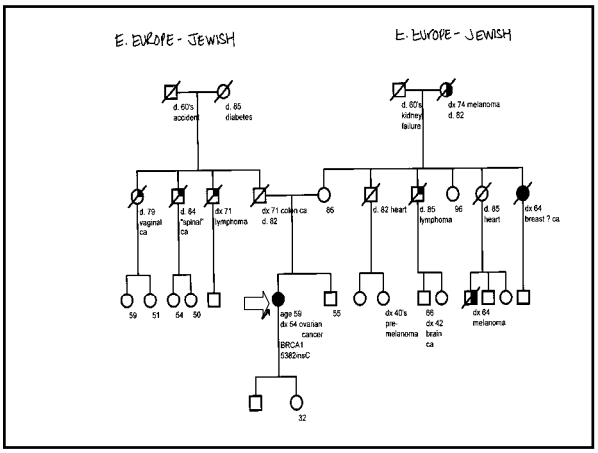
Clinical Risk Assessment

- 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**

Am. J. Human Genetics-2001, 2003, Lancet-2001

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 IIIC 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

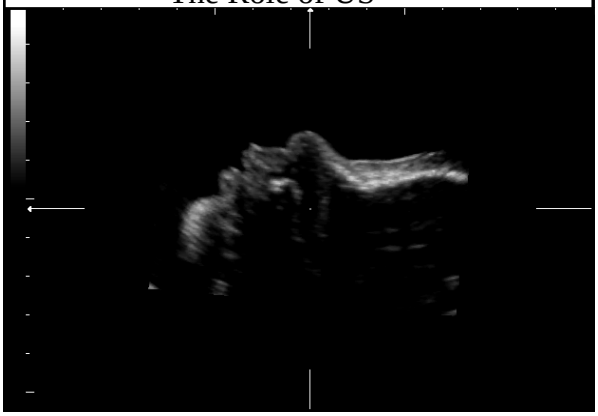
- 212 women
- 3 Gynecologic Malignancies
  - –PPC- 1 Stage IIIB
  - –FT- 1 Stage IIIA
  - –OVCA- 1 Stage IB
  - –No PPC in women s/p BSO

Cancer Detection

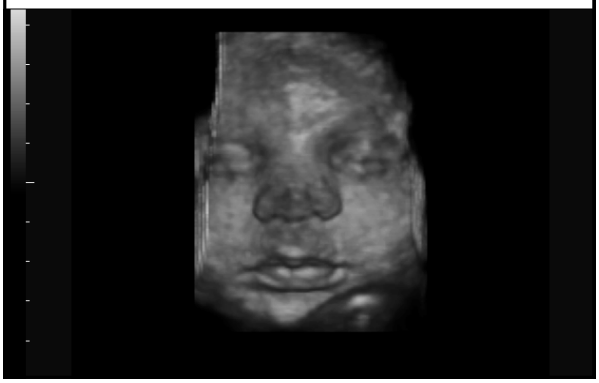
- 571 Benign
- 3 Primary Peritoneal Cancer- all Stage III
- 3 Fallopian Tube Cancer- Stage II/III(2)
- 4 Ovarian Cancer- 2- Stage I, 2- Stage III
- 10 Cancers –
  - 2- Stage I
  - 1- Stage II
  - 7- Stage III

Abdominal Ultrasound

The Role of US



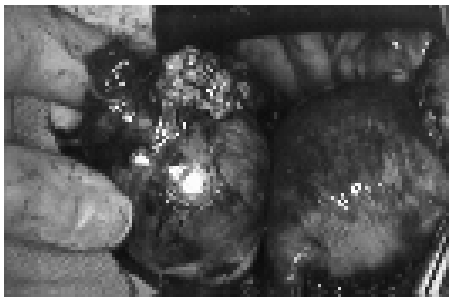
Three Dimensional Ultrasound



Recent Advances In Ultrasound

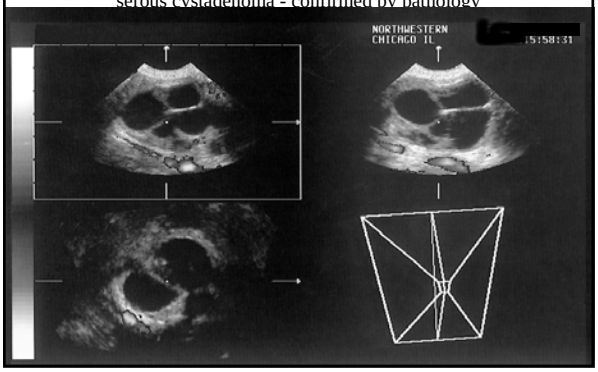
- Power Doppler Energy- improved specificity as secondary test (83-92%)
- 3-Dimensional volume acquisition and power Doppler- identifies architectural and vascular changes in observed mass, increases specificity from 54% to 75% as a secondary test
- Microvascular Imaging (MVI)- capillaries visualized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

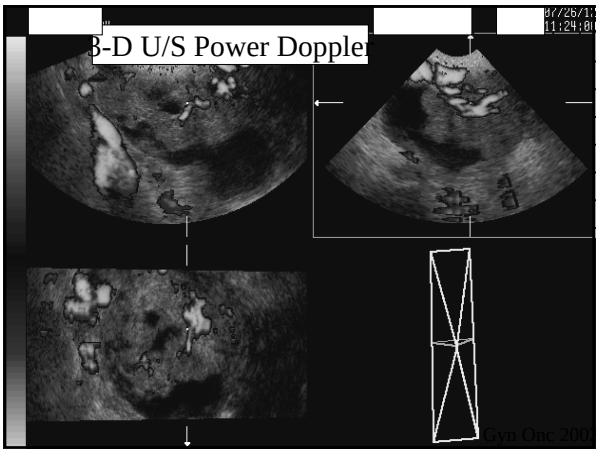
Gyn Onc 2001, Gyn Onc 2002, Lancet 2003



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

AJOG 2005

NOCEDP

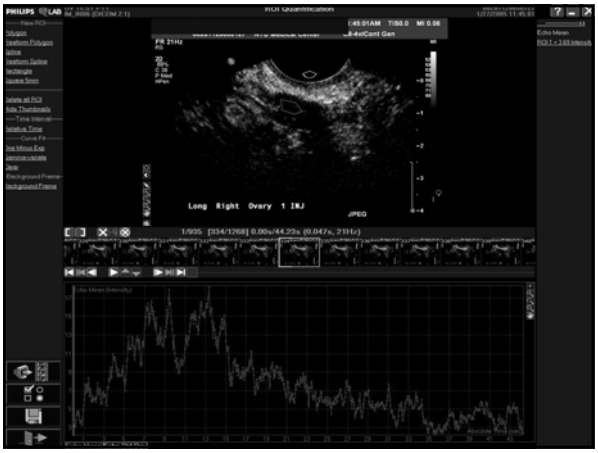
- 12 asymptomatic gynecologic cancers detected (4 fallopian tube, 4 primary peritoneal, 2 epithelial ovarian carcinoma, 2 uterine)
- all Stage III/IV (A, B, and C) except uterine (both stage1A G1)
- all normal US and PE 12 and 6 months prior to abnormal scan
- FT/PPC - normal ovaries

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

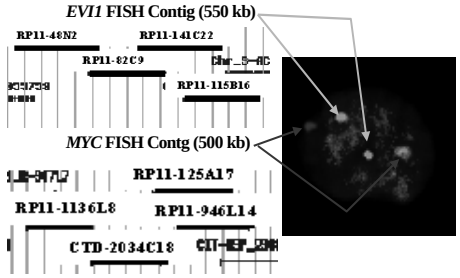


Ovarian Pap Test

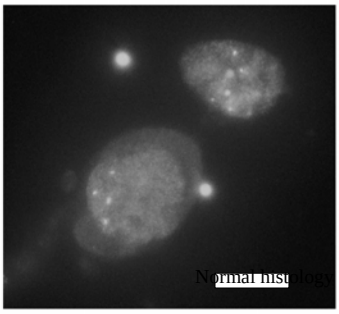
- **Minimally invasive office laparoscopy- outpatient procedure**
- **Genomics and proteomics can detect precancer/cancer years before cytology- Prevention**

Cont Ob/Gyn 2003, NEJM 2003

FISH assay: EVI1 and MYC for EOC detection



BRCA1+ Mutation with Normal Cytology



Prophylactic BSO

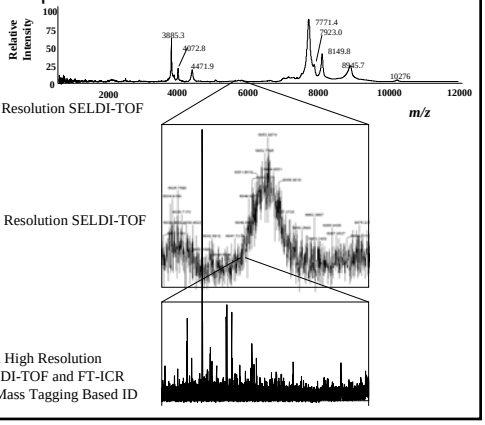
Abnormal Copy Number of EVI1 and MYC

Serum Proteomic Patterns-

Emanuel Petricoin, Lance Liotta, et al- Lancet 2002

- 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

The Rapid Evolution of MS Instrumentation

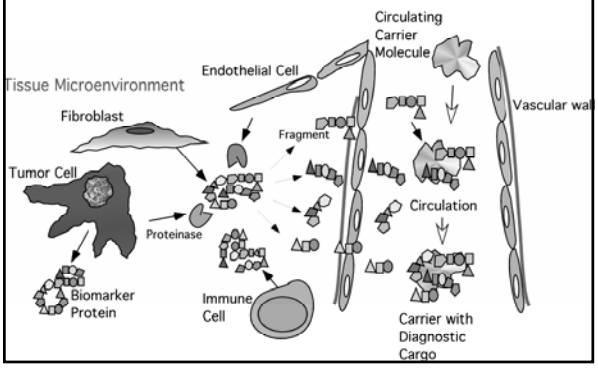


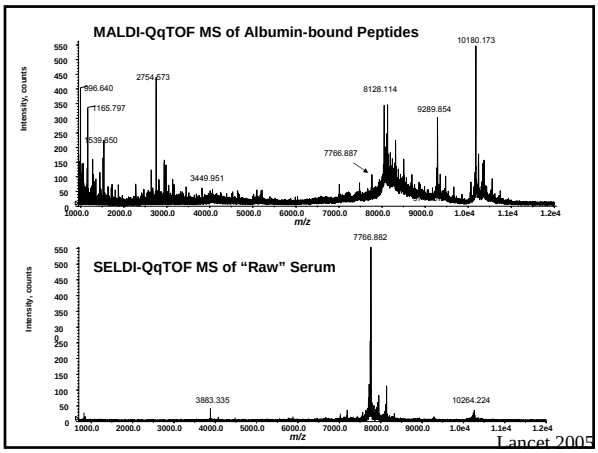
Year 2002= Low Resolution SELDI-TOF Lancet 2002

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 JNCI 2005

Biomarker Amplification and Harvesting by Carrier Molecules





Lancet 2005

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***

- 3', 5' –cyclic-GMP phosphodiesterase
- A Chain A, Crystal Structure of Human Apolipoprotein
- A Chain A, Transthyretin (Prealbumin)
- A1AG Human Alpha 1 Acid Glycoprotein 1 Precursor (AGP 1)
- A1AT Human Alpha-1-Antitrypsin Precursor, Alpha-1 Protease Inhibitor, Alpha-1-Antiproteinase
- A1BG Human Alpha 1B Glycoprotein
- A2MG Human Alpha 2 Macroglobulin Precursor
- ADP-Ribosylation Factor Binding Protein 3, golgi localized gamma ear containing, ARF binding protein 3, KIAA0154 gene product
- AF070710 envelope glycoprotein (Human Immunodeficiency Virus Type 1)
- AF077721 Pol Protein (Human Immunodeficiency Virus Type 1)
- AF094250 envelope glycoprotein (Human Immunodeficiency Virus Type 1)
- AF099171 protease (Human Immunodeficiency Virus Type 1)
- AF190128 Rev (Human Immunodeficiency Virus Type 1)
- AJ228172 gp120 (Human Immunodeficiency Virus Type 1)
- ALC1 Human Ig Alpha-1 Chain C Region
- Alpha 1 & 2 Hemoglobin, HBA Human Hemoglobin Alpha Chain
- APA1 Human Apolipoprotein A-I Precursor, Apolipoprotein A-I
- APA2 Human Apolipoprotein A-II Precursor, Apolipoprotein A-II
- APC1 Human Apolipoprotein C-I Precursor, Apolipoprotein C-I
- APC3 Human Apolipoprotein C-III Precursor, Apolipoprotein C-III
- LPHUB apolipoprotein B-100 precursor – human
- apolipoprotein D, apoD (human, plasma)
- apolipoprotein E (homo sapien)
- ASNS Human Asparagine Synthetase (glutamine hydrolyzing), TS11 Cell Cycle Control Protein
- AT Human Alpha-1-Antitrypsin (Internal Fragment)
- B Chain B, Crystal Structure of A Human Fcg
- B Chain B, Crystal Structure of S-Nitroso-Nit
- BBHU CFAB Human Complement factor B Precursor, C3/C5 Convertase, Properdin Factor B, Glycine Rich Beta Glycoprotein GBG
- BRS3 Human Bombesin Receptor Subtype 3, Uterine Bombesin Receptor
- C Chain C, Human Serum Transferrin, Recombinant
- C1HUQB Complement Subcomponent C1q Chain B Precursor
- C4HU complement precursor (validated)
- Ceruloplasmin, Ferroxidase Human
- CFA1 Human Complement Factor I Precursor, C3B
- Clusterin, Complement Cytolysis Inhibitor (CLI), SP-40, Sulfated Glycoprotein 2, testosterone Repressed Prostate Message 2

JNCI-2005, JBC-2005

Clinically Relevant Biological Markers for Early Detection

- **Lysophospholipds (LPA) -LC/MS/MS**
- **Growth factors (p110, p60)**
- **Proteases (MMPs, Kallikreins)**
- **Proteomics- SELDI/ MALDI-TOF, ABI QqTOF, ESI-MS**

Female Patient 2002, Cancer Res 2002, Lancet 2002, Nature 2003, Can Res 2004, Nature Med 2004, Gyn Onc 2004

What is required for the clinician and patient to achieve optimal healthcare?

New Paradigm for Cancer Detection

Asymptomatic Patient

⇩

Serum/Plasma Protein and Lipid Analysis

⇩

Diagnostic Imaging-US MVI

⇩

Ovarian Pap Test/ Biopsy- Cytopathology- Gene/Protein Profile

⇩

Detection of Early Stage Disease

Multiplexed serum assay for early detection of ovarian cancer

Anna Lokshin, PhD

OVARIAN CANCER: STATISTICS

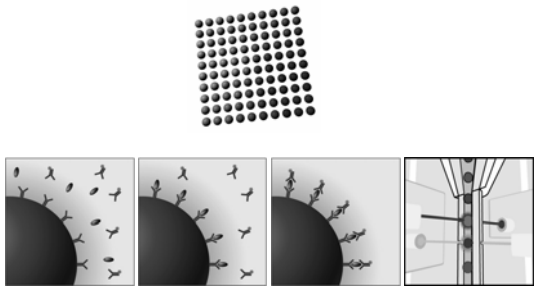
- **Fifth most common cancer in the US**
- **Accounts of 4% of all cancers in women**
- **Causes more deaths than any other gynecological cancer**
- **About 23,000 new cases of ovarian cancer and 14,000 deaths each year in the US**
- **The 5-year survival for patients with clinically advanced ovarian 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

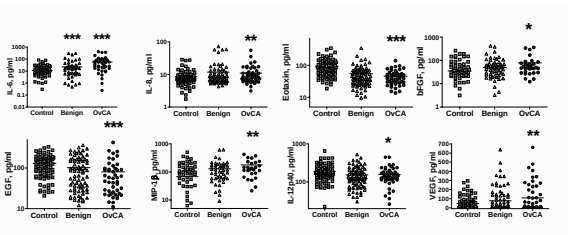
PATIENTS

Patient Group	Age	Histologic Types
Control N=85	Range 33-76 Median 46	
Early Stage Ovarian Cancer N=61	Range 34-88 Median 46	Papillary serous carcinoma Carcinoma, endometroid Carcinoma, mucinous Carcinoma, poorly differeniated Adenocarcinoma, serous Carcinoma, clear cell
Benign Tumors N=75	Range 35-87 Median 44	Adenofibroma, serous Brenner tumor Crystadenofibroma, serous Cyst, paratubal Cyst, serous Cyst, simple Cystadenofibroma, serous Cystadenoma, mucinous Cystadenoma, serous Endometriosis Fibrosis Ovary benign Mucinous benign

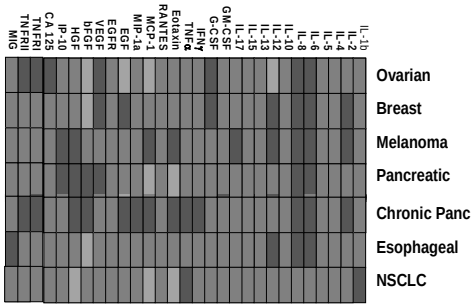
INITIAL SCREENING: LUMINEX ANALYTES

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, TNFR I, TNFR II, IFNγ, GM-CSF, G-CSF **Chemokines:** RANTES, MIP-1a, MIP-1b, MCP-1, Eotaxin, MIG **Growth and angiogenic factors:** EGF, EGFR, VEGF, bFGF, HGF, VEGF, NGF
Panel II	**Cancer Antigens:** CA 125, CA 15-3, CEA, AFP, 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, EGFR, 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**

Selected panel of ovarian cancer markers



CYTOKINE PROFILES ARE CANCER-SPECIFIC



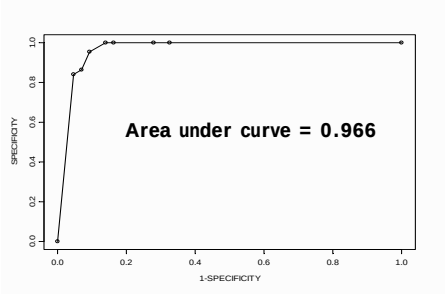
CLASSIFICATION RESULTS FOR INDIVIDUAL MARKERS (Controls vs. OC)

Cytokine	%Correctly Classified	%Sensitivity	%Specificity
EGF	80.5	84.1	76.7
VEGF	73.6	79.5	67.4
MCP	78.2	84.1	72.1
IL-6	85.1	84.1	86.0
IL-8	79.3	88.6	69.8
IL-12	73.6	72.7	74.4
G-CSF	58.6	40.9	76.7
CA125	85.1	95.5	74.4

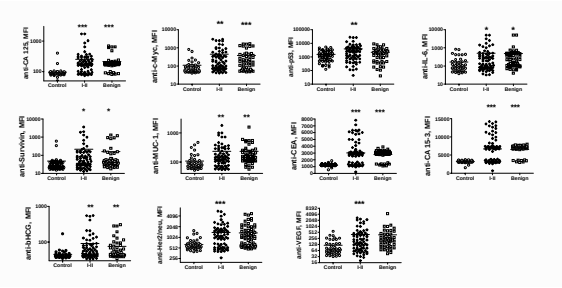
Optimal model for cytokines/CA 125

CLASSIFICATION RESULTS	OvCA vs. Control CA 125+IL-6+IL-8+EGF+VEGF	OvCA vs. Benign CA 125+G-CSF+IL-6+EGF+VEGF
%Correctly Classified	93.0	80.2
%Sensitivity	95.0	84.1
%Specificity	91.0	75.7

ROC curve. Early stage ovarian cancer vs. healthy controls



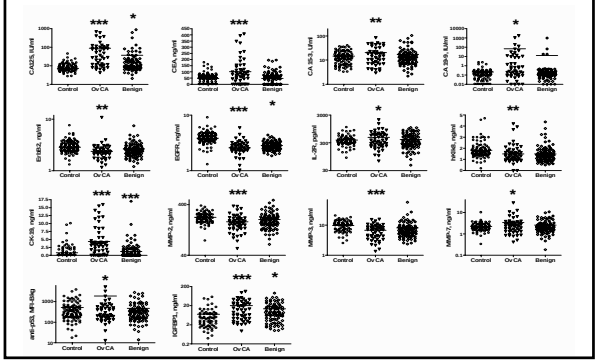
SERUM LEVELS OF CIRCULATING ANTIBODIES



Optimal model for circulating antibodies

CLASSIFICATION RESULTS	OvCA vs. Control CA 15-3+IL-8+surv+p53+c-myc	OvCA vs. Benign CA 15-3+CES+p53+IL-6+c-myc+bHCG+EGF+IL-8
%Correctly Classified	97.5	88.0
%Sensitivity	94.3	94.3
%Specificity	100.0	79.0

SERUM LEVELS OF CANCER MARKERS



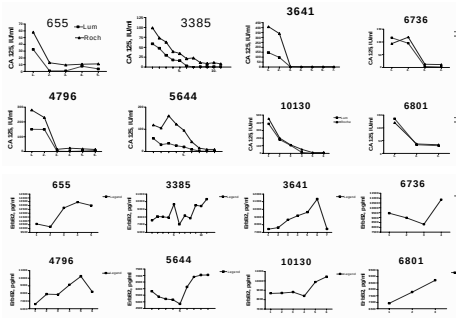
Optimal model for cancer antigens

CLASSIFICATION RESULTS	Cancer vs. Control (Panel of 17)	Cancer vs. Benign
%Correctly Classified	96.3	
%Sensitivity	90.0	
%Specificity	99.0	

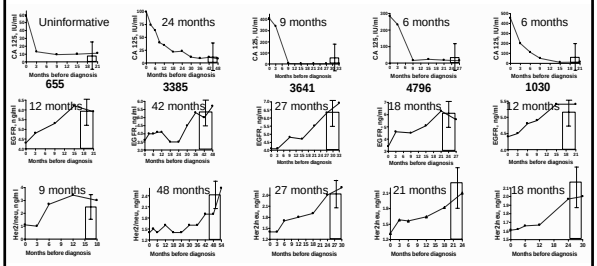
CONCLUSIONS I

- LabMAP technique allows for high-throughput multimarker analysis of serum markers in ovarian cancer
- Combination of several markers allows for higher sensitivity and specificity that 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

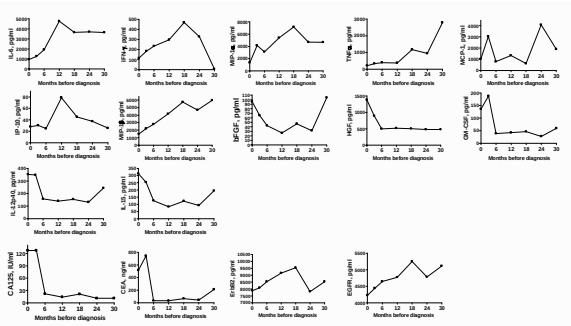


Changes in concentrations of CA 125, EGFR and Her2/neu in women before clinical diagnosis of ovarian cancer (prospective study)

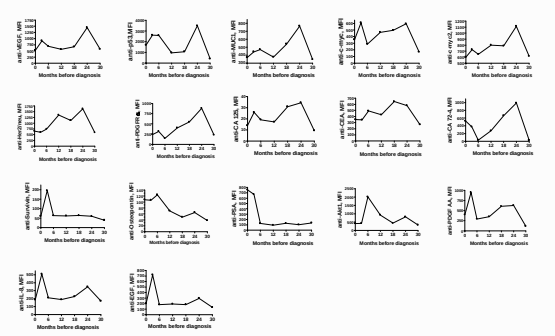


1. "0" corresponds to the point of clinical diagnosis
2. Numbers in red indicate lead time before clinical diagnosis
3. Bars indicate levels in healthy women ± SD
4. Serum samples were obtained from Dr. Ian Jacobs (University College of London, London, UK)

Velocities of cytokines and cancer markers



Velocities of circulating antibodies



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
Adele 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

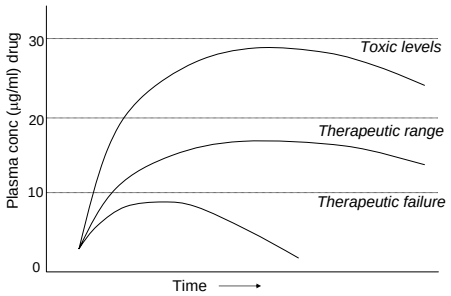
University College of London, UK
Ian Jacobs, MD
Usha Menon, MD

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



Patient Response to Medicine Varies

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

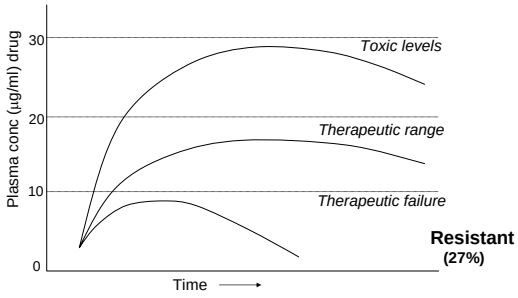
What determines drug disposition?

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)

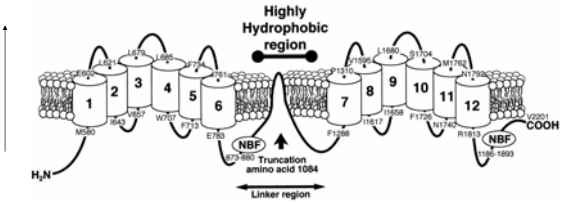
73% of patients respond
27% of patients do NOT respond



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



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.

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

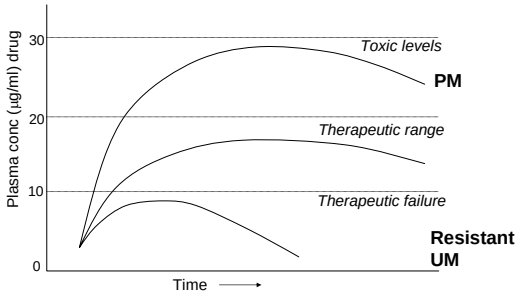
Cytochrome P450 Enzymes

- ✓ Superfamily of Phase I enzymes
- ✓ $\geq$ 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?

Gene	PM	IM	EM	UM
CYP2D6	10%	35%	48%	7%
CYP2C9	4%	38%	58%	N/A
CYP2C19	3-21%	N/A	79 Š 97%	N/A

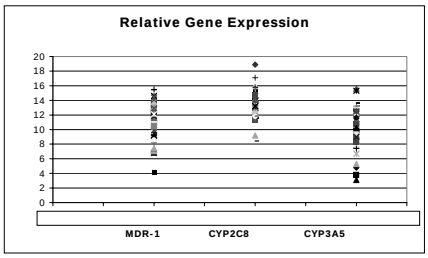
PM = poor metabolizer
IM = intermediate metabolizers
EM = extensive metabolizers
UM = ultrametabolizers



Do Ovarian Cancers Express PK-related Genes?

Gene	Expression
MDR-1	47/48 (98%)
CYP2C8	33/48 (69%)
CYP3A4	4/48 (8.3%)
CYP3A5	42/47 (89%)

Expression Levels Vary Widely



Relationship between expression and grade

	MDR-1[1]	CYP2C8	CYP3A5
Grade 1 (N=10)	221 +/- 436	13.8 +/- 15.3	108 +/- 97
Grade 2/3 (N=26)	634 +/- 2010	57 +/- 129	447 +/- 1159
p value	0.865	0.105	0.653
Grade 3 (N=16)	322 +/- 507	68 +/- 161	632 +/- 1456
Grade 1/2 (N=20)	173 +/- 325	27 +/- 38	201 +/- 343
p value	0.319	0.334	0.263

[1]Gene expression values are an average +/- standard deviation of the calculated relative expression.

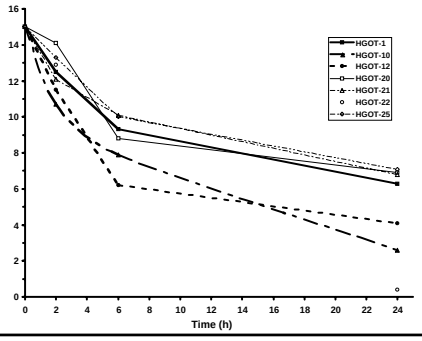
Relationship between expression and stage

	MDR-1	CYP2C8	CYP3A5
Stage1 (N=10)	119 +/- 138	14.5 +/- 15	291 +/- 484
Stage 2/3 (N=26)	290 +/- 481	56.6 +/- 129	435 +/- 1158
p value	0.120	0.0565	0.609
Stage 3 (N=20)	343 +/- 539	72.8 +/- 143	519 +/- 1312
Stage 1/2 (N= 16)	119 +/- 139	9.95 +/- 13.1	236 +/- 386
p value	0.096	0.066	0.369

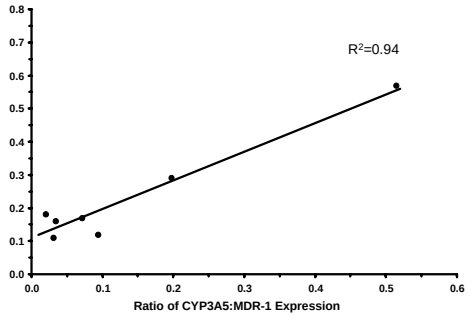
Relationship between expression and histologic type

	MDR-1	CYP2C8	CYP3A5
Endometroid (N=8)	177 +/- 175	19.5 +/- 24.5	711 +/- 1735
Others (N=28)	260 +/- 468	52 +/- 125	305 +/- 718
p value	0.453	0.202	0.536
Papillary serous (N=18)	269 +/- 454	74 +/- 152	317 +/- 832
Others (N=18)	214 +/-393	16 +/- 19.9	484 +/- 1211
p value	0.706	0.128	0.641

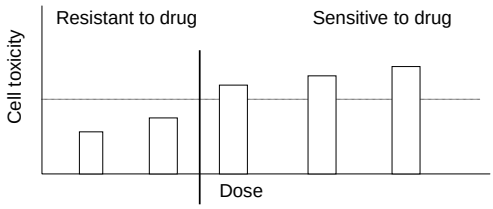
Metabolism Does Occur in Tumor Cells



Rate of metabolism relative to expression



Do levels predict chemosensitivity?

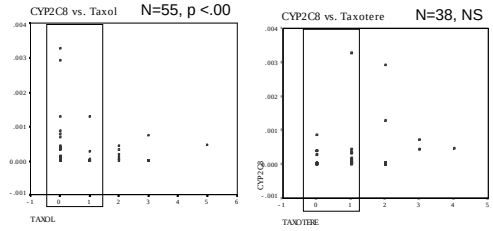


***Ex Vivo* Chemosensitivity Assay: PTI**

Do levels predict chemosensitivity?

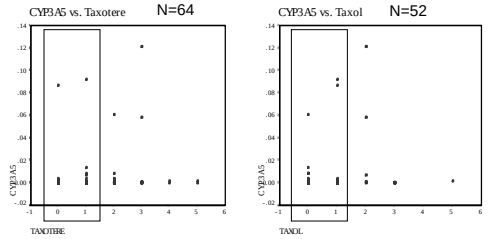
Transporters:	ABCC1 ABCB1 (MDR-1) ABCG2
Cytochrome P450	CYP2C8 CYP3A4 CYP3A5
Drugs	Taxol Taxotere Etoposide, Topotecan Gemcitabine, 5-FU

CYP2C8 expression correlates with response to paclitaxel

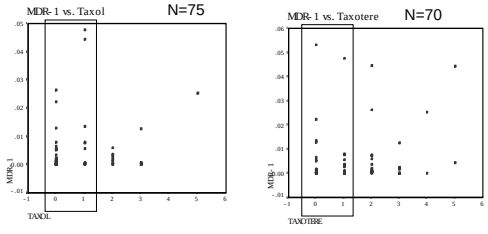


Resistant ⟶ Sensitive

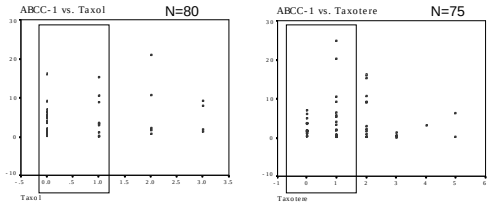
CYP3A5 expression does not correlate with response to docetaxel



MDR-1 expression does not correlate with response to docetaxel or paclitaxel

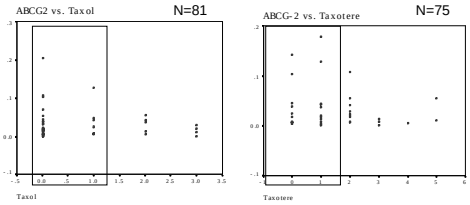


Other Transporters - ABCC1 (MRP1)



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

MWRI
Jackie Jones-Laughner
Janiene Patterson
Mary Strange

UPCI
William Zamboni, Ph.D.
Sandra Strychor

MWH
Joseph Kelley, M.D.
Bob Edwards, M.D.
Paniti Sumkumvanich, M.D.
Tom Krivak, M.D.

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

Precision Therapeutics Inc.
Holly H. Gallion, M.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

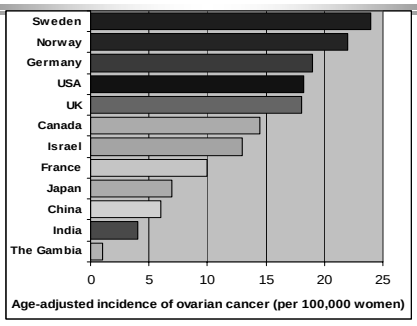
GOG 170D

- **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
GEOGRAPHIC VARIATION



Adapted from Parkin, et al. Cancer in Five Continents, *IARC*, Lyon, 1992.

Reducing Ovarian Cancer Mortality
HORMONES: RISK MODIFICATION

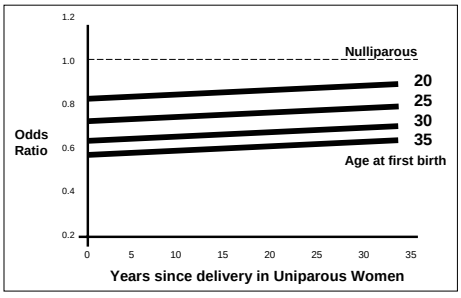
- **Pregnancy**
- **Oral contraceptive exposure**

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
INFLUENCE OF A SINGLE PREGNANCY



From Adami H-O, et al. *Lancet* 1994; 344:1250-1254

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

Whittemore: Am J Epidemiol 136:1184

Reducing Ovarian Cancer Mortality
PARITY AND ORAL CONTRACEPTIVES

Relative Risk of Ovarian Cancer

	Years of OC Use	
	0	≥4
Parity		
0	1.00	0.41
≥3	0.41	0.17

Hartge et al., Obstet Gynecol 1994; 84:760

Beyond Treating the Patient

Patricia Goldman
Ovarian Cancer National Alliance

Ovarian Cancer National Alliance

- Umbrella Organizaton
  - 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

Peritoneal Immunotherapy

October 24-25, 2005

Ralph S. Freedman
UT MD Anderson Cancer Center

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

- Armstrong DK, Bundy BN, Baergen R, et al: Randomized phase III study of intravenous (IV) paclitaxel and cisplatin versus IV paclitaxel, intraperitoneal (IP) cisplatin and IP paclitaxel in optimal stage III epithelial ovarian cancer (OC). A GOG trial, Proc. ASCO, 21:201a, 2002
- Alberts DS, Liu PY, Hannigan EV, et al: Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. New Engl J Med 335:1950-1955, 1996
- Markman M, Bundy BN, Alberts DS, et al: Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal displatin in small-volume stage III ovarian carcinoma. An intergroup GOG study, J Clin Oncology 19:1001-1007, 2001

	IP Chemotherapy	IP Immunobiotherapy
Applications	Consolidation 1st line combination	Consolidation Adjuvant to chemo
Target	Surface & floating tumors	Surface & floating tumors immune cells/?other
Target Dose	Sub MTD	Range below MTD
R_x objectives	Max. intratumoral levels	Optimum biologic effect
	⇓ systemic toxicity	⇓ systemic toxicity
Molecule size	Small (< 500 daltons)	Large: 15 to >50 kd.
Half life (bolus)	Short	Long: 20 + h
Penetration distance	1 mm = Maximum (platinum)	Paradoxical increase

Affected by diffusivity (D), rate of capillary removal (k) & other factors: non-tumor tissue uptake, convection, lymphatic flow, affinity (mABs)

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, Willemse, Frasci (1985-90)**
Viral oncolysate	**Freedman (1984-89)**
rIFNα+CDDP/Carbo	**Nardi, Berek, Markman, Frasci (1990-94)**
rIFNγ	**D'Aquisto (1988)**
rIL2	**Chapman, Melioli, Edwards (1988-97)**
rIL2 +LAK	**Stewart, Steiss (1990)**
Bifunctional a/b+rIL2+ T-cells	**Canevari (1995)**
rIL2+TIL	**Freedman (1994)**
rIFNγ	**Pujade-Lauraine (1996)**
rIFNγ +rIL2	**Freedman (1998)**
rIL12	**Lenzi (2002)**
FLT3-L	**Freedman (2002)**

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**
- **rIFNγ – 23% CPR (incl. tumors > 1cm)**

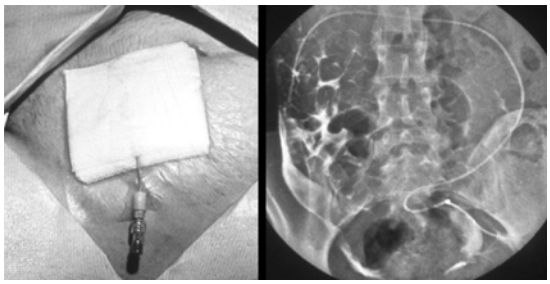
Clinical Toxicity: **Generally acceptable – dose/schedule dependent**

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

Percutaneous Angiocatheter



Catheter *in situ* Fluoroscopic localization

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)

- **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**

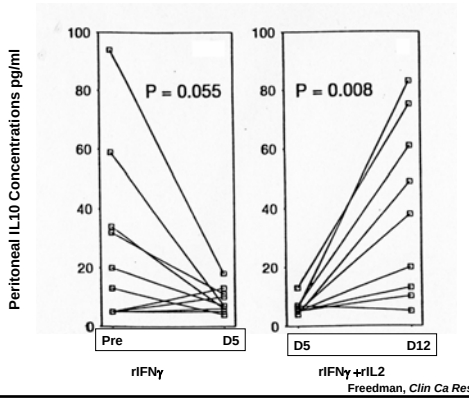
Pharmacokinetics (PK) & Pharmacodynamics (PD) in IP Trials

- 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***

Effects of IP rIFNγ Sequenced with IP rIL2 on PF IL10 Levels



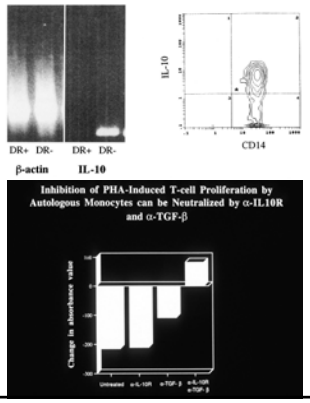
Freedman, *Clin Ca Res*, 2000

Cytokine /Chemokine Transcripts in a Responding EOC Patient

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

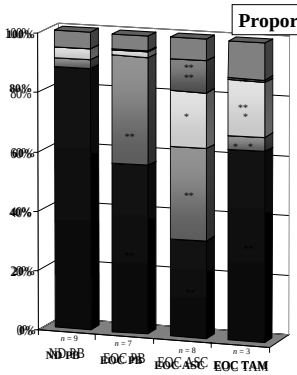
Cytokine/ Chemokine	Pretreatment (agm/l)	Posttreatment (agm/l)	
		Week 1	Week 3
IFN-γ	< 2,470	38,660	15,970
IL-2	0	0	0
IL-10	3,190	830	500
IP10	520	620	810

IL-10 Production in HLA$^-$DR Monocytes



A Loercher, et al, *J Immunol*, 1999

Proportions of MO/MA Subpopulations



- $CD14^{dim}$
- $CD14^{bright}CD16^+HLA\text{-}DR^-$
- $CD14^{bright}CD16^+HLA\text{-}DR^+$
- $CD14^{bright}CD16^-HLA\text{-}DR^-$
- $CD14^{bright}CD16^-HLA\text{-}DR^+$

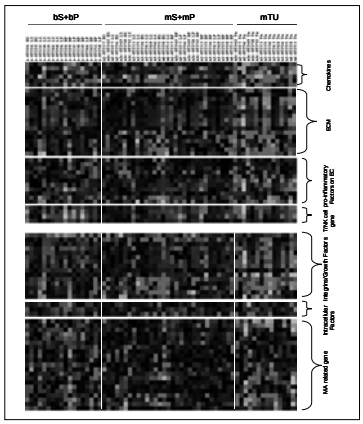
** p<0.01 vs. ND PB
* p<0.05 vs. EOC PB
** p<0.01 vs. EOC PB
* p<0.05 vs. EOC ASC

Adapted from Okrent I and Freedman R. Abstract #1259 in *Proceedings of American Association for Cancer Research 95th Annual Meeting;* March 2004.

ADCC & Phagocytosis

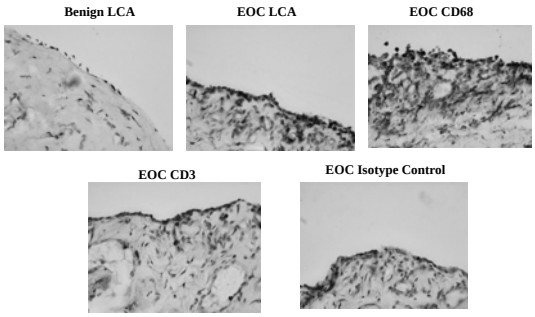
Cytokine Production by MDM

Supervised Cluster Analysis of Genes Differentially Expressed



E Wang et al, 2005

MO/MA & T-Cell Infiltration of Peritoneum



Peritoneal Monocytes

Ascitic MO/MA

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/leukotrines produced by tumor or inflammatory cells
- Alteration adherance 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
- Clonally expanded sequenced T-cell transcripts (Pappas, *Cellular Immunol*, 2005)
- 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 proliteration (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 CD3 ζ chain (P)
  IFNγ or IL12 suppress IL10 production
- Antibodies/antisense (gene silencing) —
  anti-TGF neutr a/b
  anti-IL10 Rc a/b
- $CD4^+$ $CD25^+$ depletion—anti-CD25 mAb

What constitutes MRD?

Potential Targets for Peritoneal & Ascitic MO/MA

MO/MA	Trabectedin induces apoptosis in MDM & induced ↓MO & inhibits CCL2 & IL6 (Allavena et al Ca Res. 2005)
Surface Molecules	CD163-mediates: IL10 release (Demidora 2004) MA targeted photodynamic Rx
Secreted Products	CXCL8 – proangiogenic & increas. capillary permeability Humanized mAb (Huang S AM J. Path. 2002
Intracellular	P38 MAPK – induces TNFα, IL1, IL6, IL8, COX2, Collagen I (Nobel, Science 2004)
Others	Vascular adhesion molecule Leukotrienes

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.washignton.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"

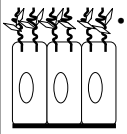
Anda Vlad MD, PhD
Department of Immunology
University of Pittsburgh
School of Medicine

MUC 1 (1989-2005)

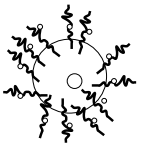
Overexpressed on all human adenocarcinomas: ovary, endometrium, breast, pancreas, colon, lung, prostate, head and neck, etc…, as well as on multiple myelomas and some B cell lymphomas

>83% of all human tumors

NORMAL → TUMOR

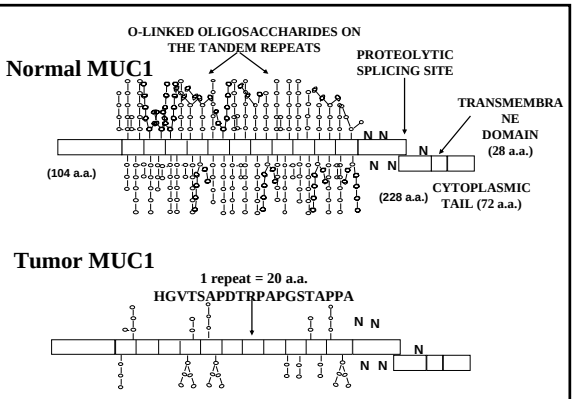


- **Loss of polarized expression**
- **Over-expression**



- **Underglycosylation:**
  T and Tn tumor carbohydrate antigens

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

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

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

Department of Immunology

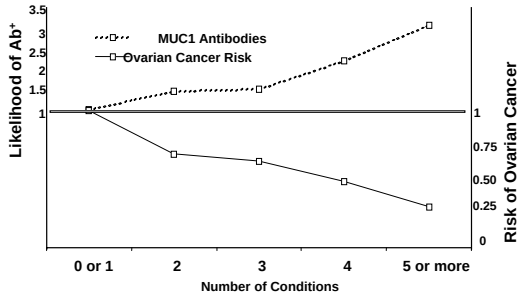
Anti-MUC1 Antibodies by Number of Conditions

	Positive for Antibodies N (%)	Negative for Antibodies N (%)	RR (95% CI)
0-1	38 (24.2)	119 (75.8)	1.00
2	76 (32.9)	155 (67.1)	1.44 (0.91, 2.29)
3	59 (34.1)	114 (65.9)	1.51 (0.92, 2.46)
4	46 (43.0)	61 (57.0)	2.20 (1.28, 3.76)
5 or more	19 (51.4)	18 (48.6)	3.11 (1.47, 6.55)

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

Department of Immunology

Anti-MUC1 antibodies by index of number of conditions and risk for ovarian cancer



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

Using vaccination to boost anti-MUC1 immunity

Department of Immunology

MUC1 peptide vaccine based on the tandem repeat is immunogenic and safe

- **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, PhaseI/II in resected pancreatic cancer prior to standard therapy (12 patients)**
   100mer MUC1 peptide on DC

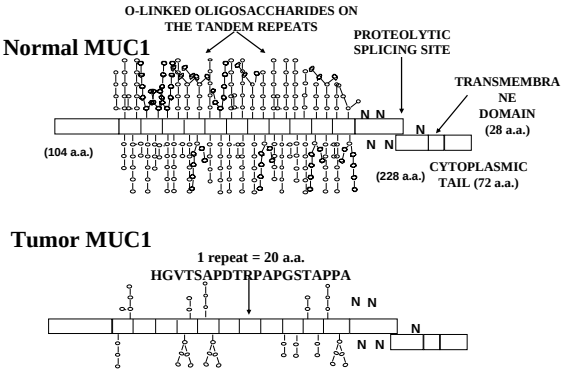
Department of Immunology

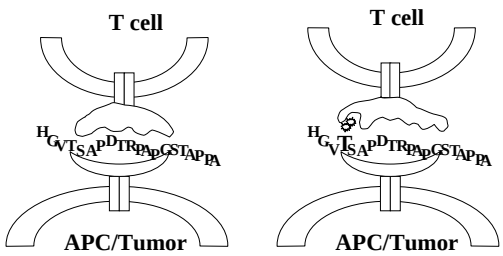
Is it possible to do better?

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

$(AHGVTSAPDTRPAPGSTAPP)_5$
100mer

GalNAc GalNAc GalNAc
$(AHGVTSAPDTRPAPGSTAPP)_5$
Tn100mer

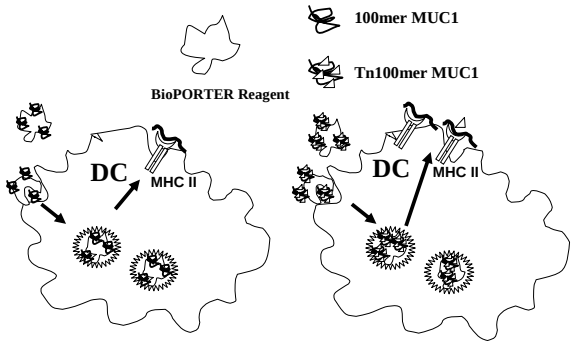




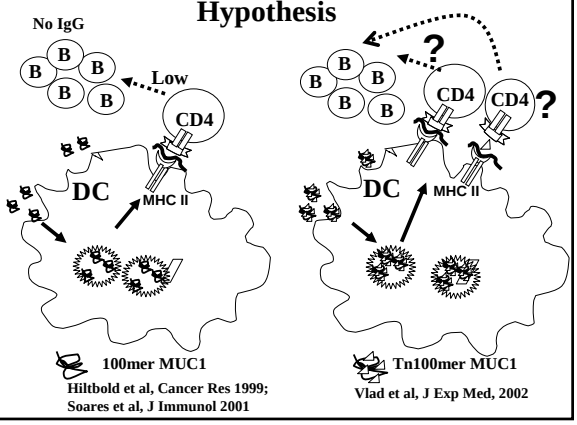
Vlad, A. et al, J. Exp. Med. 2002

Department of Immunology

DC-MUC1 Vaccine



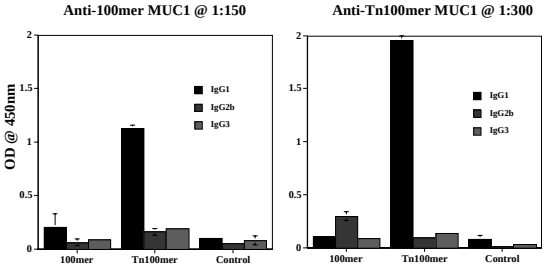
Hypothesis



Hiltbold et al, Cancer Res 1999;
Soares et al, J Immunol 2001

Vlad et al, J Exp Med, 2002

Anti-MUC1 IgG antibody responses in DC-MUC1 vaccinated mice



Department of Immunology

Anti-MUC1 IgG antibody responses in DC-MUC1 vaccinated mice

ELISA plates coated with:

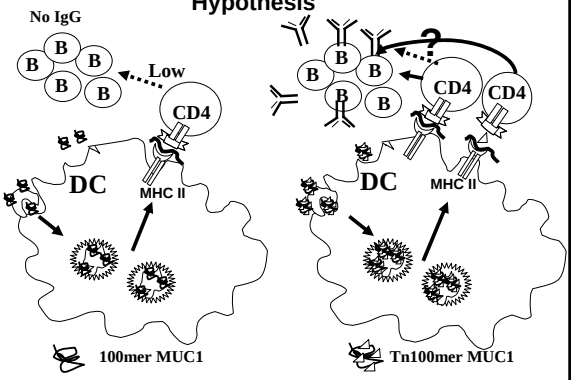
Sera from mice vaccinated with:	100mer	Tn100mer
DC-BP-100mer	-	-
DC-BP-Tn100mer	+	++

End-point titer 1:1,000

Summary of T cell results

T cells from mice vaccinated with:	T helper responses to: 100mer	Tn100mer
DC-BP-100mer	+	-
DC-BP-Tn100mer	++	++

Hypothesis



Is it possible to do even better?

Department of Immunology

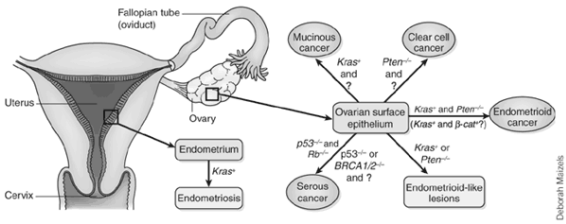
Department of Immunology

A relationship between inflammation and cancer

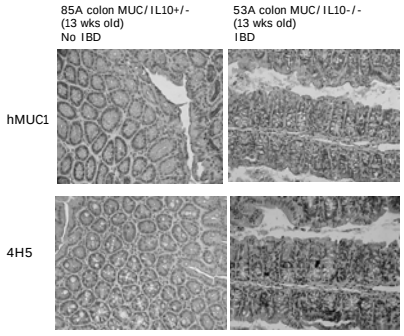
- Hashimoto's thyroiditis and thyroid cancer
- Chronic pancreatitis and pancreatic cancer
- Chronic airway inflammation and lung cancer
- Inflammatory bowel disease (IBD) and colon cancer
- Endometriosis and endometrioid ovarian cancer

Exploring the relationship between MUC1, endometriosis and endometrioid ovarian cancer

Gynecologic diseases get their genes.



Martin M Matzuk. *Nature Medicine* 11, 24 - 26 (2005)



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

Department of Immunology

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

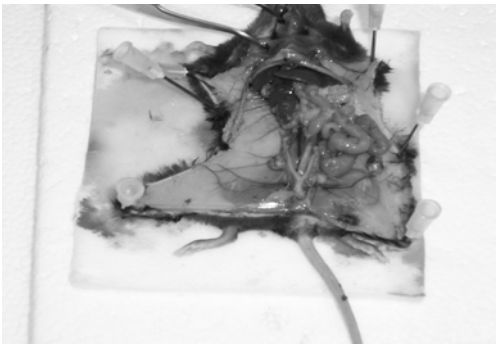
- Does the immune system have the capacity to recognize EOC?
- Preliminary results from a phase I trial.
- Describe the repertoire of CT antigens and define potential targets for vaccine therapy.

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.

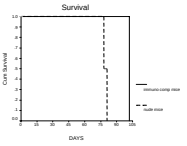
The critical role of T cells in EOC: syngeneic murine model using ID8



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

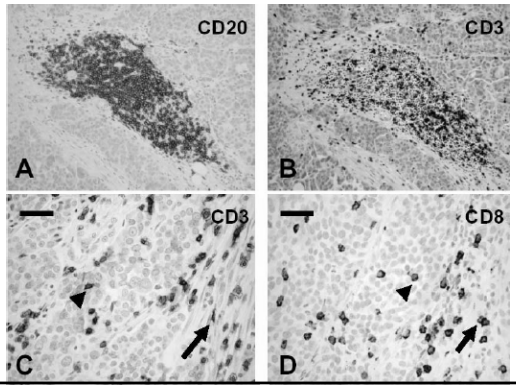
ID8 murine
EOC cell line:IP

Immunocompetent: survival > 120 days.

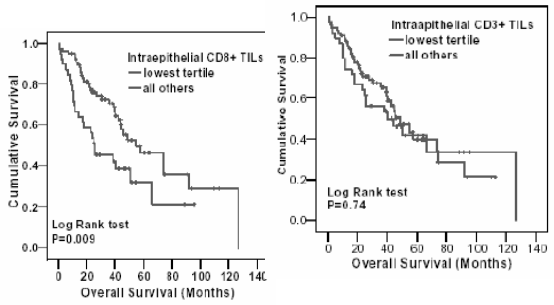


Nude (athymic) mice: survival 70 days

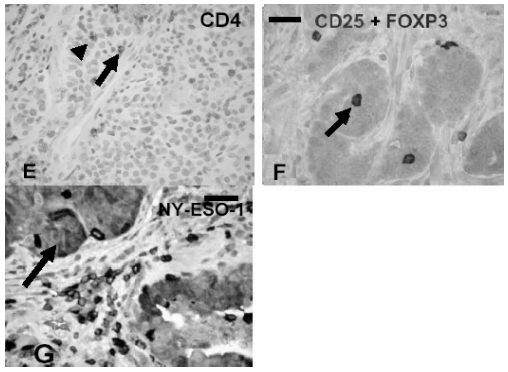
CD20+, CD3+ and CD8+ TILs in ovarian cancer



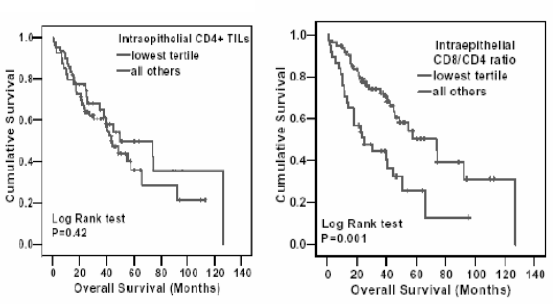
Prognostic significance of TILs in EOC: 1



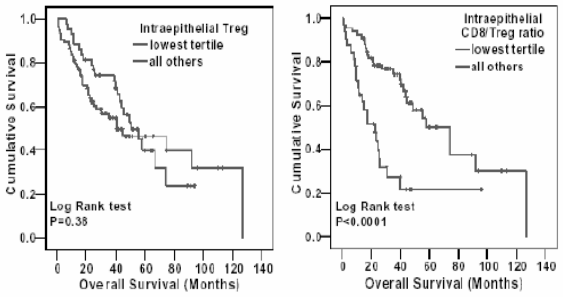
CD4+, CD25+FOXP3+ TILs in ovarian cancer



Prognostic significance of TILs in EOC: II



Prognostic significance of TILs in EOC: III



Conclusions: TILs in Epithelial Ovarian Cancer

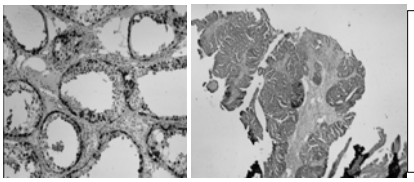
- High Intraepithelial CD8+TILs is associated with improved survival: > 50% improvement in overall survival (55 versus 26 months; Hazard ratio = 0.33, P=0.0003).
- High intraepithelial CD8+/CD4+ T cell ratio (>3.1) is associated with improved survival: (74 months versus 25 months; Hazard ratio 0.30, 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)

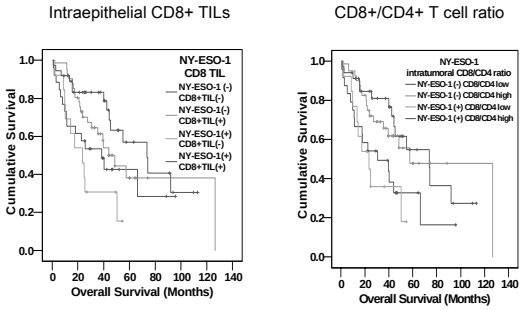


NY-ESO-1 mRNA expression

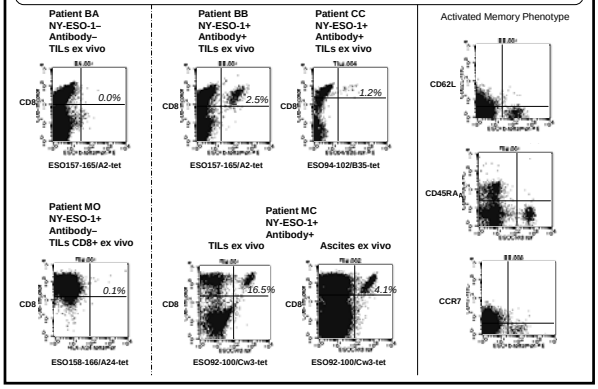
•Melanoma	35%
•Breast	30%
•Liver	30%
•Lung	25%
•Colon	< 5%

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

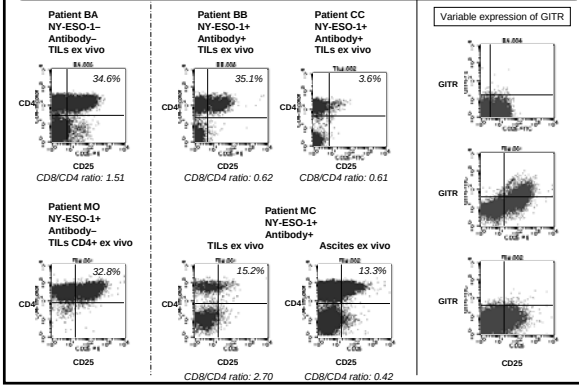
Prognostic significance of TILs in EOC: IV



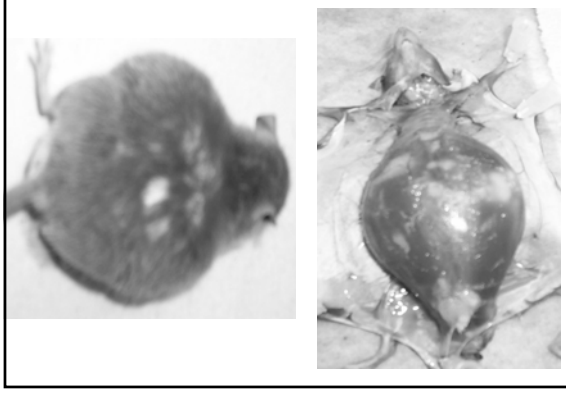
Presence of CD8+ T Cells Specific for NY-ESO-1 in Ovarian Tumor Infiltrating Lymphocytes (Direct *ex-vivo* tetramer analysis)



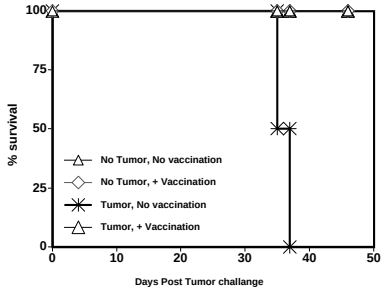
Variable presence of CD4+ CD25+ T Cells in Ovarian Tumor Infiltrating Lymphocytes



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



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



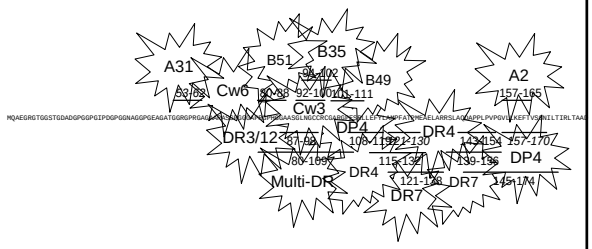
A pilot clinical trial of NY-ESO-1DP4 peptide in patients with EOC whose tumors express NY-ESO-1

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

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



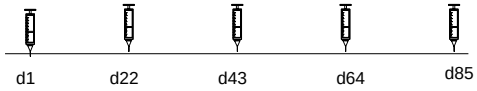
Eligibility criteria

- 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.
- Karnorfsky performance status >70%
- Life expectancy > 6 months.

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-1expressing tumors

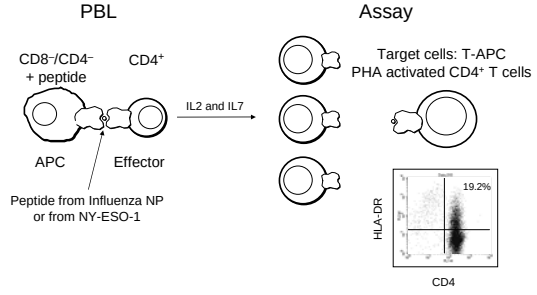


Maximum of three courses (each consisting of 5 vaccinations given at 3 weekly intervals, providing patient remains NED)

Monitoring

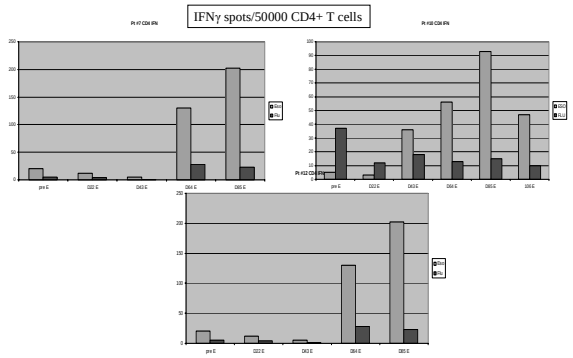
- Toxicity
  - NCI criteria
- Immunological monitoring:
  - DTH: Recall antigens, DP4, ESO1b
  - Humoral immunity
  - CD4+ and CD8+ T cell responses : Tetramer, IFN-γ ELISPOT
  - Time to disease progression.

Presensitization with HLA class II peptides



NY-ESO-1 specific CD4+ T cell responses (IFNγ 8/14; IL5 8/9)

IFNγ spots/50000 CD4+ T cells



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%)

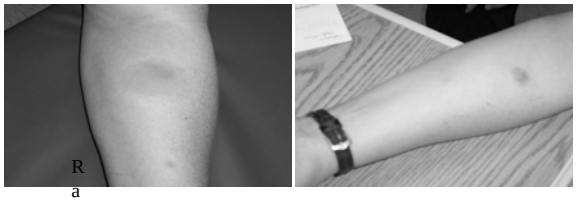
- *More time points remain to be tested

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

Age (median)	**60 yrs**
Performance status	**>70%**
Recurrences prior to vaccine therapy	
None	**10 (55%)**
Yes	**8 (45%)**
No of prior lines of chemotherapy	
1	**10 (55%)**
2-8	**8 (45%)**
Serous histology	**17**
Grade III	**16**
Stage of primary tumor	
Stage IIIC	**16**
Stage IIC	**2**

DTH Responses: Patient 7

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

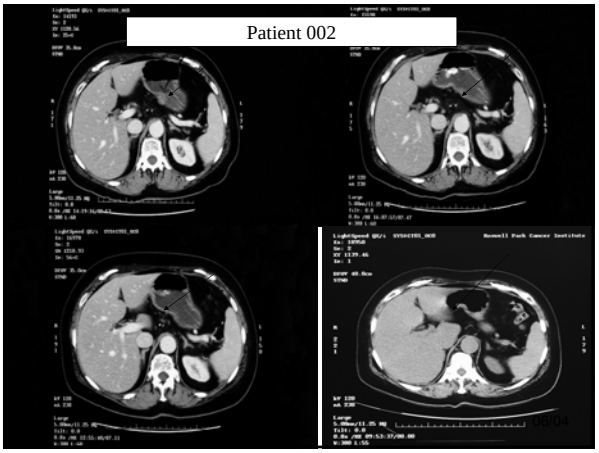


R
a
n

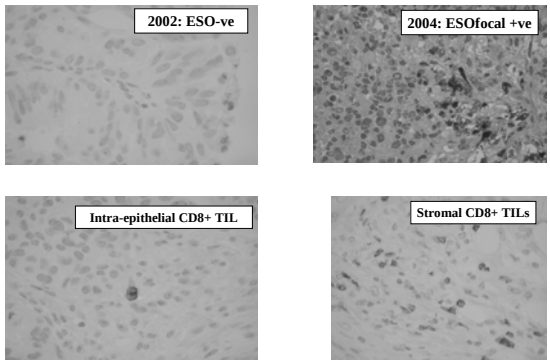
Summary of clinical results

DTH to recall antigens at baseline	**13 (72%)**
Tetanus	**7**
Tetanus + Candida	**5**
Candida	**1**
Disease status at study entry	
NED	**11 (61%)**
Minimal residual disease	**3**
Non-target lesion	**4**
Current Status	
NED	**9**
Progression	**3**
Recurrence	**4**
DOD	**2**

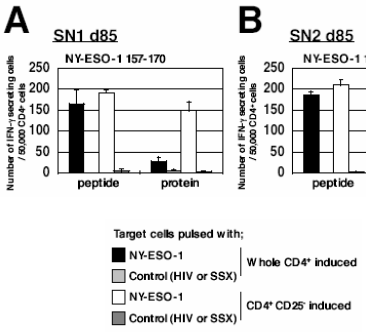
Patient 002



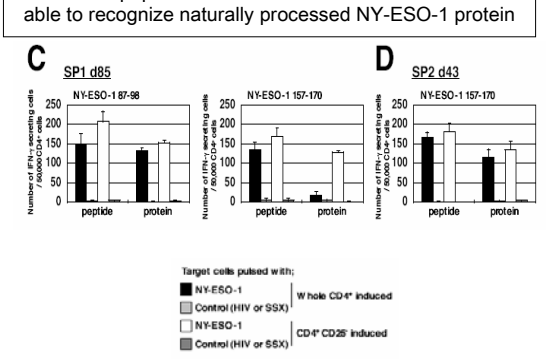
Patient 002: Antigen expression and TILs



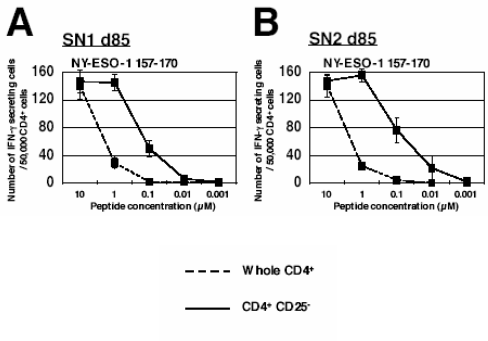
NY-ESO-1 peptide vaccine induced CD4+ T cells are not able to recognize naturally processed NY-ESO-1 protein



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



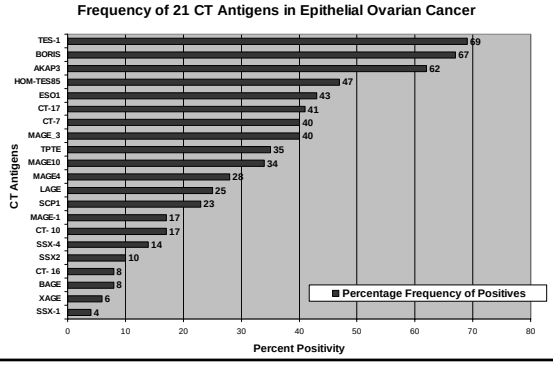
Tregs suppress high avidity NY-ESO-1 specific CD4+ Tcells



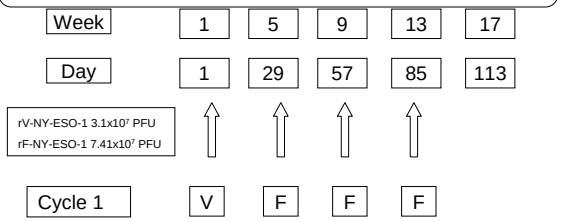
Challenges and Lessons

- 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

Repertoire 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



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

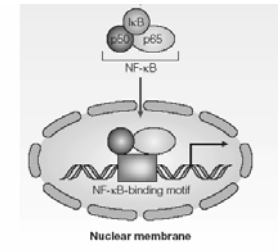
TLR-4 signaling promotes tumor growth and paclitaxel chemo-resistance in ovarian cancer

Thomas J. Rutherford
Yale University School of Medicine
2005

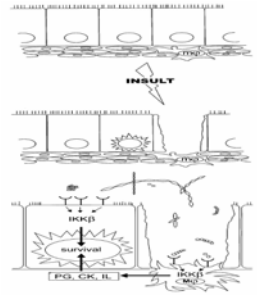
Inflammation —?→ **Cancer**

Chemo-resistance

NF-kB



Akira, 2004



(Clevers, 2004)

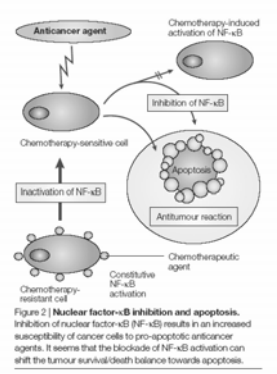
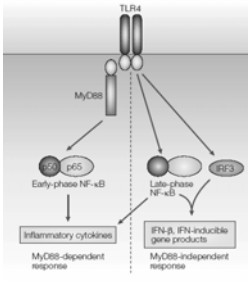


Figure 2 | **Nuclear factor-κB inhibition and apoptosis.** Inhibition of nuclear factor-κB (NF-κB) results in an increased susceptibility of cancer cells to pro-apoptotic anticancer agents. It seems that the blockade of NF-κB activation can shift the tumour survival/death balance towards apoptosis.

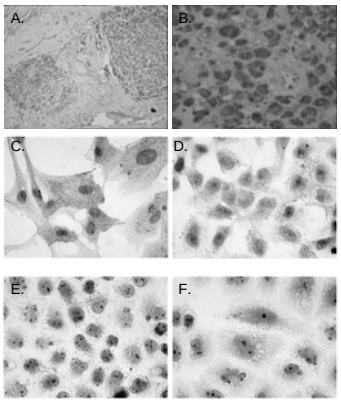
(Nakanishi et al, 2005)

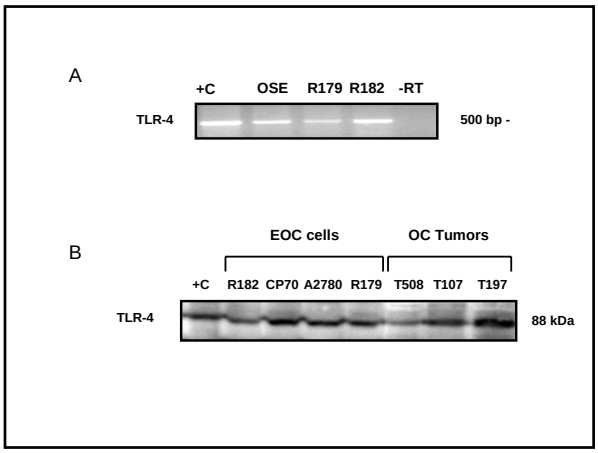
TLR signaling

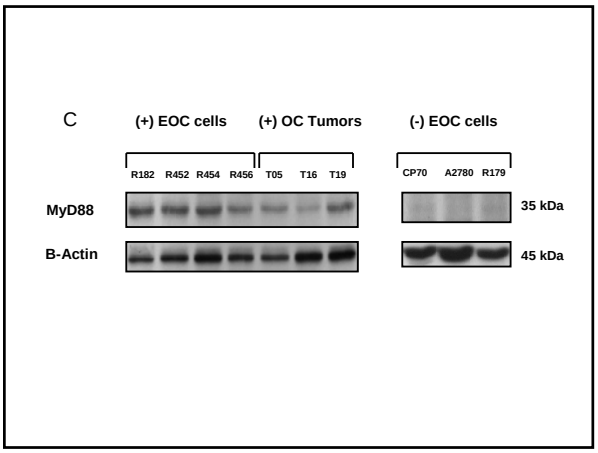


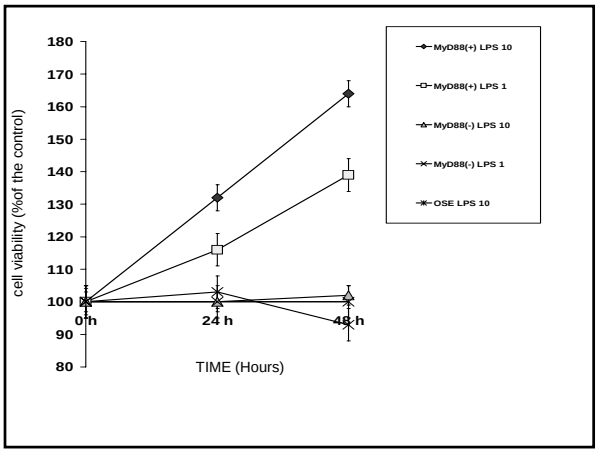
TLR4	Lipopolysaccharide
	Taxol
	Fusion protein
	Envelope protein
	Heat-shock protein 60*
	Heat-shock protein 70*
	Type III repeat extra domain A of fibronectin*
	Oligosaccharides of hyaluronic acid*
	Polysaccharide fragments of heparan sulphate*
	Fibrinogen*

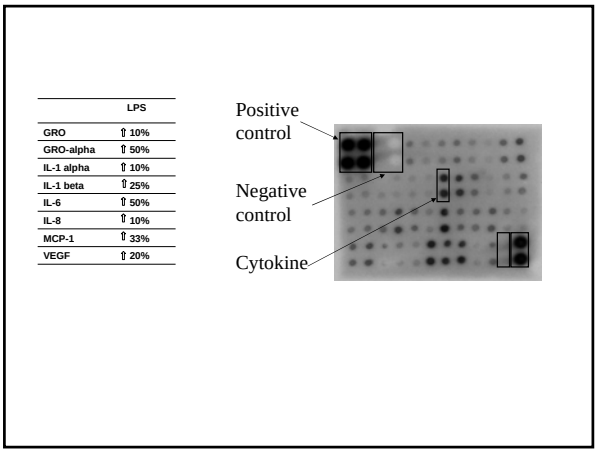
Akira and Takeda, 2004

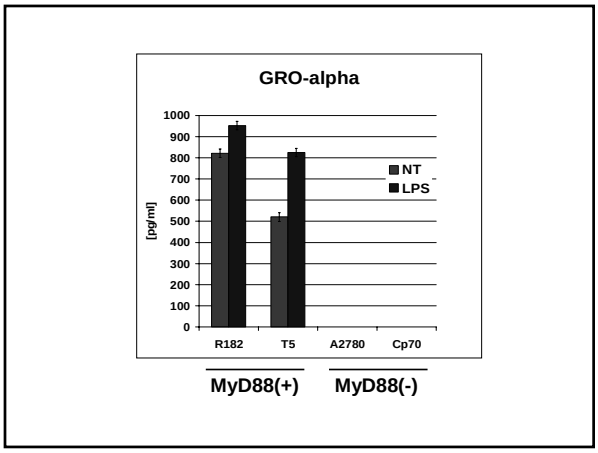


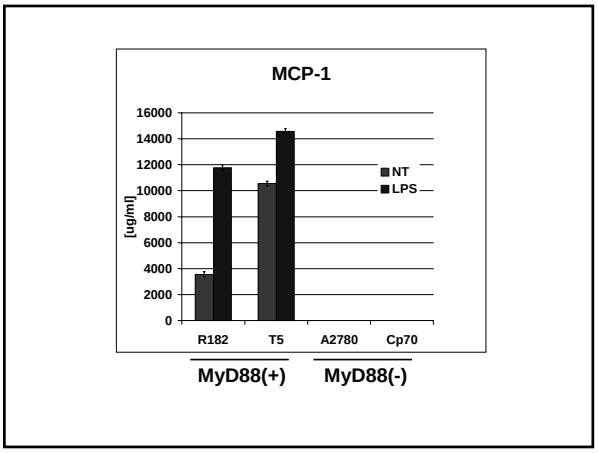

A
+C
OSE
R179
R182
-RT
TLR-4
500 bp -
B
EOC cells
OC Tumors
+C
R182
CP70
A2780
R179
T508
T107
T197
TLR-4
88 kDa

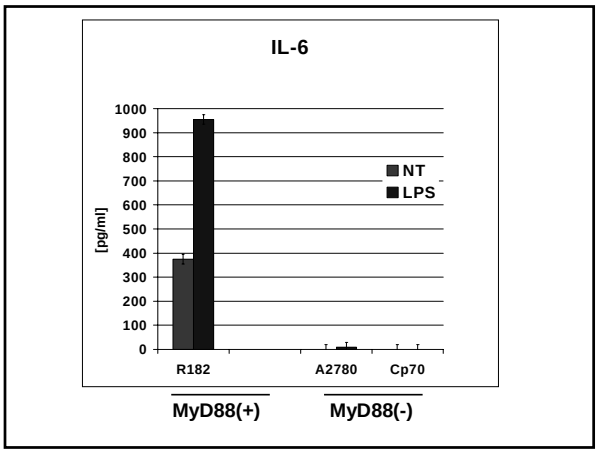

C
(+) EOC cells
(+) OC Tumors
(-) EOC cells
R182
R452
R454
R456
T05
T16
T19
CP70
A2780
R179
MyD88
B-Actin
35 kDa
45 kDa

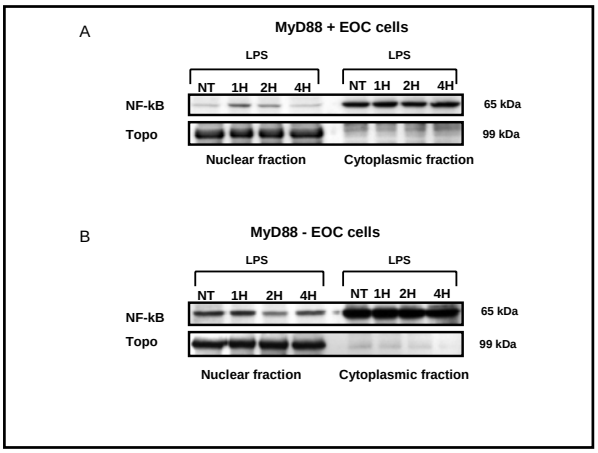

cell viability (%of the control)
TIME (Hours)
0 h
24 h
48 h
MyD88(+) LPS 10
MyD88(+) LPS 1
MyD88(-) LPS 10
MyD88(-) LPS 1
OSE LPS 10

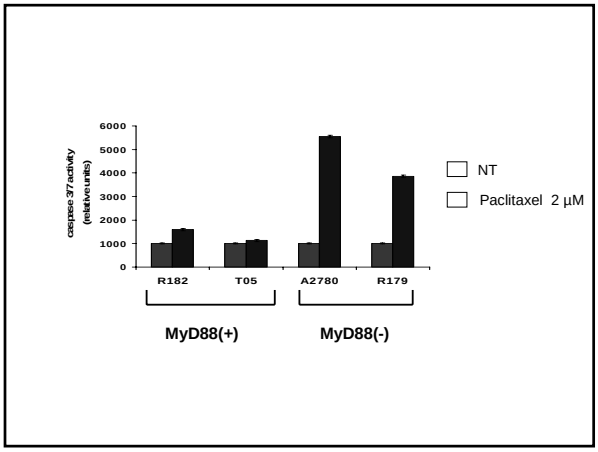

LPS
GRO ⇧ 10%
GRO-alpha ⇧ 50%
IL-1 alpha ⇧ 10%
IL-1 beta ⇧ 25%
IL-6 ⇧ 50%
IL-8 ⇧ 10%
MCP-1 ⇧ 33%
VEGF ⇧ 20%
Positive control
Negative control
Cytokine

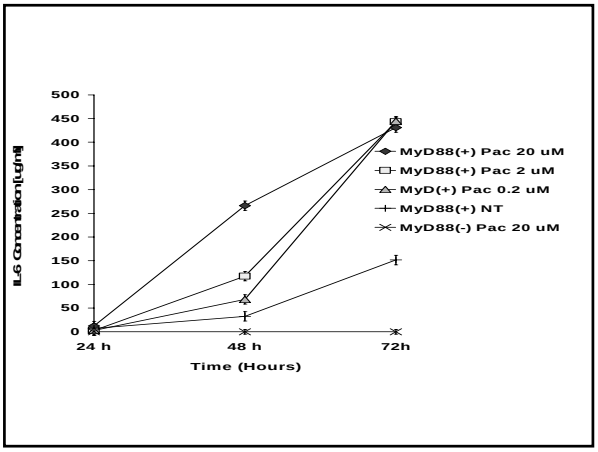

GRO-alpha
[pg/ml]
NT
LPS
R182
T5
A2780
Cp70
MyD88(+)
MyD88(-)

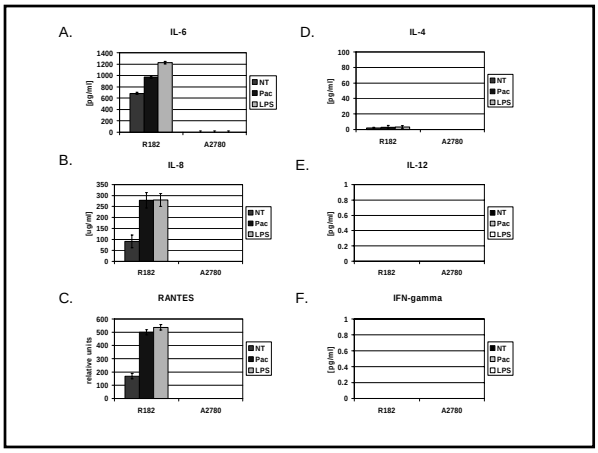

MCP-1
[ug/ml]
NT
LPS
R182
T5
A2780
Cp70
MyD88(+)
MyD88(-)

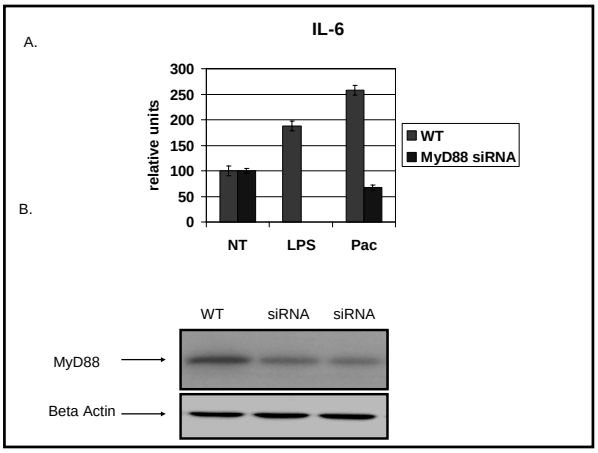

IL-6
[pg/ml]
1000
900
800
700
600
500
400
300
200
100
0
NT
LPS
R182
A2780
Cp70
MyD88(+)
MyD88(-)

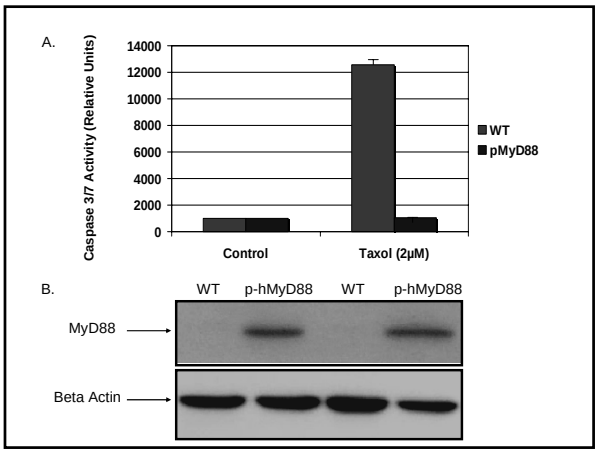

A
MyD88 + EOC cells
LPS
LPS
NT 1H 2H 4H
NT 1H 2H 4H
NF-kB
Topo
65 kDa
99 kDa
Nuclear fraction
Cytoplasmic fraction
B
MyD88 - EOC cells
LPS
LPS
NT 1H 2H 4H
NT 1H 2H 4H
NF-kB
Topo
65 kDa
99 kDa
Nuclear fraction
Cytoplasmic fraction

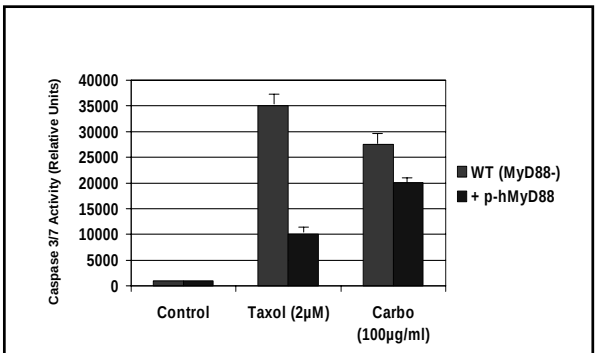

caspase 3/7 activity (relative units)
6000
5000
4000
3000
2000
1000
0
NT
Paclitaxel 2 µM
R182
T05
A2780
R179
MyD88(+)
MyD88(-)

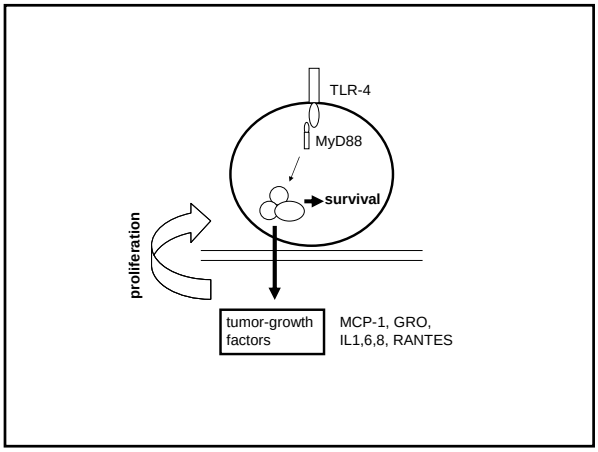

IL-6 Concentration(pg/ml)
500
450
400
350
300
250
200
150
100
50
0
MyD88(+) Pac 20 uM
MyD88(+) Pac 2 uM
MyD(+) Pac 0.2 uM
MyD88(+) NT
MyD88(-) Pac 20 uM
24 h
48 h
72h
Time (Hours)


A.
IL-6
D.
IL-4
B.
IL-8
E.
IL-12
C.
RANTES
F.
IFN-gamma
NT
Pac
LPS
R182
A2780


A.
IL-6
relative units
300
250
200
150
100
50
0
WT
MyD88 siRNA
NT
LPS
Pac
B.
WT
siRNA
siRNA
MyD88
Beta Actin







Acknowledgements

Research Staff
- Gil Mor
- Ayesha Alvero
- Irene Visintin
- Vikki Abrahams
- Shawn Chavez
- Roy Chen
- Paula Aldo
- Serena Chen

Clinical Staff
- Peter Schwartz
- Masoud Azodi
- Michael Kelly
- Jessica McAlpine
- Dan Silasi
- Lisa Baker
- Martha Luther

Oregovomab: Challenges, Lessons, and Opportunities

Christopher F. Nicodemus, MD FACP
SVP Clinical Research & Development
Unither Pharmaceuticals, Inc.

Monoclonal antibodies specific for self antigens can trigger antigen-specific cellular immunity

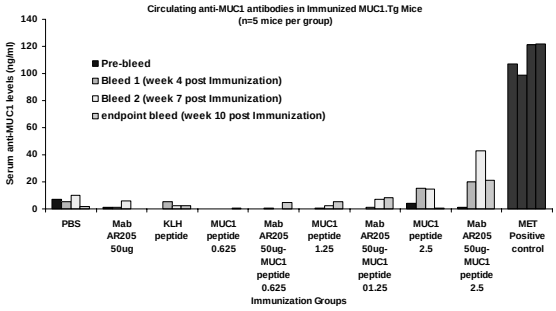
- The observation represents an alternative immunotherapeutic strategy to standard "vaccine approach"
  - Review evidence from MUC1 and CA125 systems
- Consider clinical development strategies for ovarian cancer immunotherapy

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

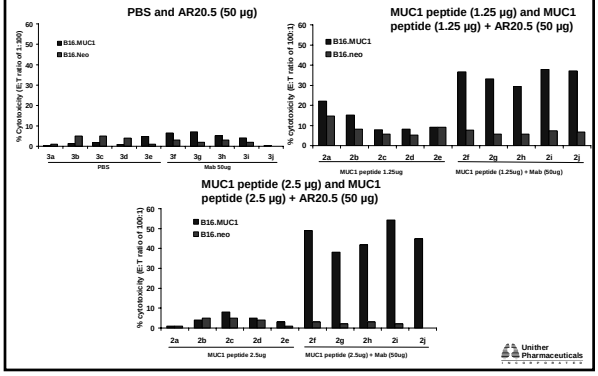
Complex-Induced B Cell Responses to MUC1

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

de Bono JS, et al. Annals of Oncology 15:1825-1833, 2004.

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

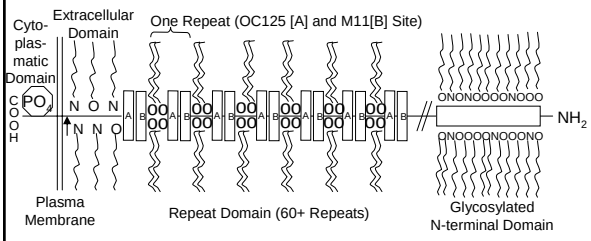
Treatment Group	Patient No.	IFN-γ ELISPOT (spots/10^5 cells)[a]	
		Baseline	Post-Injection[b]
1 mg	AA006	433	89
2 mg	AA008	0	30
	AA009	0	144
	AA011	0	0
	AA012	0	0
	AA014	0	500
4 mg	AA016	140	800
	AA017	1030	>2800[c]
	AA019	160	0
	AA020	0	0

[a] Number of spots counted in ELIPSOT assays performed after *in vitro* stimulation of peripheral blood lymphocytes obtained from the patients at baseline or after treatment
[b] Maximum number of spots counted at any time after the first injection of MAb-AR20.5
[c] Number of spots exceeded amount that can be quantified by the Zeiss Image Analyzer

de Bono JS, et al. Annals of Oncology 15:1825-1833, 2004.

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



O'Brien TJ, et al. Tumor Biology 23:154-169, 2002.

T Cell Epitopes on CA125

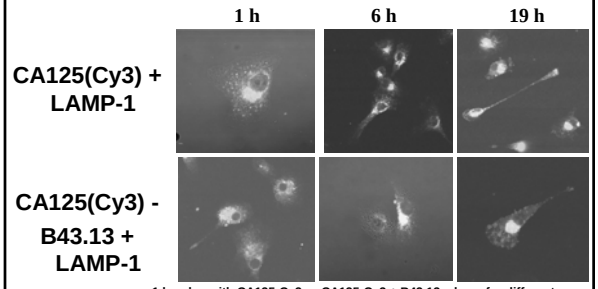
- Full sequence evaluated for HLA-A2 epitopes
- Candidate peptides being identified
- Pentamer reagent development ongoing

Monoclonal Antibody B43.13

Oregovomab MAb-B43.13 (OvaRex®)

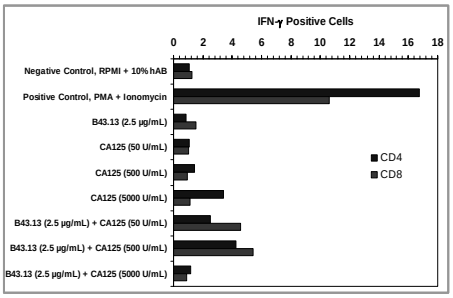
- Murine IgG_{1k} specific for CA125
- Formulated for 2 mg infusions (50 mL over 20 minutes)
- Complexes CA125 in circulation
- Does not have direct effects (ADCC or CDC)
- Results in altered antigen processing of CA125 and subsequent lymphocyte response

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



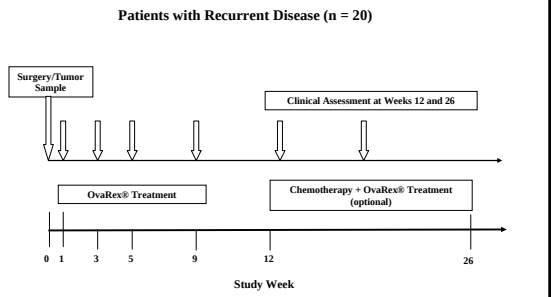
1 h pulse with CA125-Cy3 or CA125-Cy3 + B43.13, chase for different time points, fixation and stain with anti-LAMP-1-FITC

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

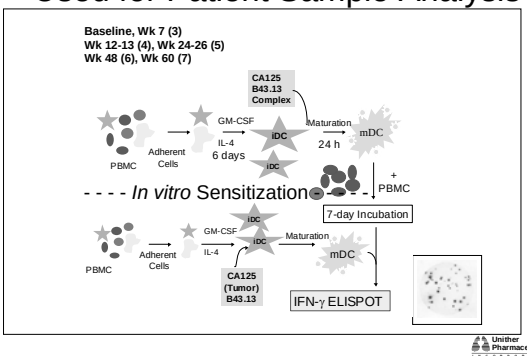


Two rounds of *in vitro* stimulation

Study OVA-Gy-12 - Design

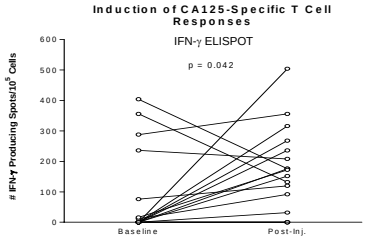


Optimized IFN-γ ELISPOT Assay Used for Patient Sample Analysis



CA125-Specific T Cells Induced with OvaRex

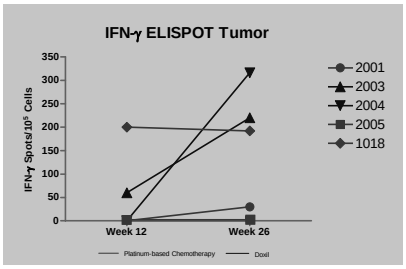
OVA-Gy-12



p value derived from Wilcoxon signed rank test
Post-injection value represents the maximum response detected at any time point after the first OvaRex® injection

T Cell Responses Maintained in Presence of Chemotherapy

OVA-Gy-12

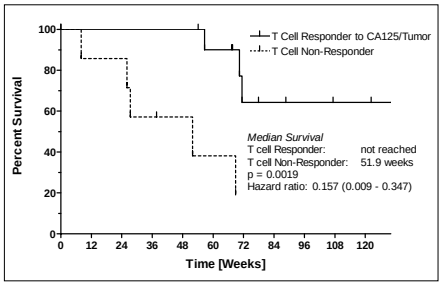


Chemotherapy between Week 12 and 26

2001: 6 cycles Carboplatin/Taxol; 2003: 6 cycles Doxil; 2004: 5 cycles Doxil; 2005: 4 cycles Cisplatin
1018: 6 cycles Carboplatin/Taxol

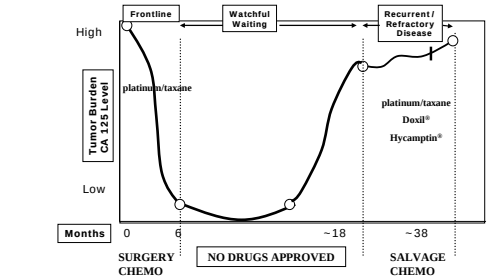
CA125/Tumor Specific T Cell Induction Associated with Significant Survival Advantage

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)

Berek JS, et al. J Clin Oncol 22(17):3507-3516.

Unither Pharmaceuticals

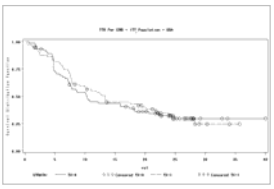
Successful Front-Line Therapy Population

- 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

Unither Pharmaceuticals

Study OVA-Gy-07: Results
Kaplan-Meier Analysis of Time to First Event (Disease Relapse or Death)

All Patients (ITT; n = 145)



Successful Front-Line Therapy Patients (n = 67)

All Patients (ITT):

- OvaRex® (n=73) median TTR = 13.3 months vs Placebo (n=72) median TTR = 10.3 months (p=0.71)

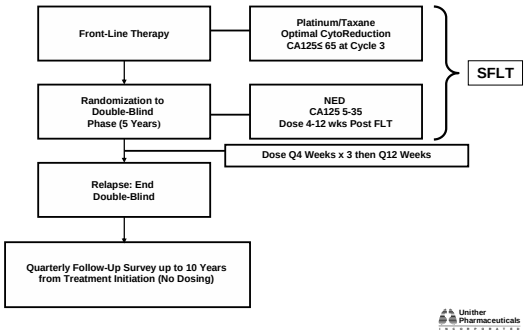
Successful Front-Line Therapy Patients (U.S.):

- OvaRex® (n=34) median TTR = 24.0 months vs Placebo (n=33) median TTR = 10.8 months (p=0.06)

Berek JS, et al. J Clin Oncol 22(17):3507-3516.

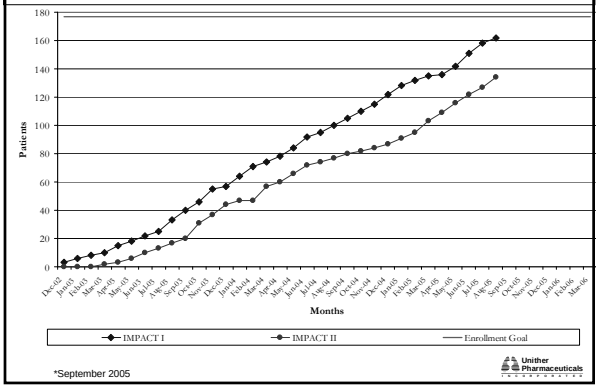
Unither Pharmaceuticals

IMPACT I/II Program
Two Phase III Protocols; 354 Patients Total



Unither Pharmaceuticals

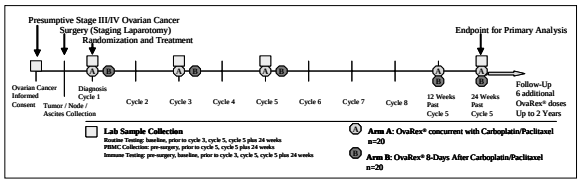
IMPACT I & II Enrollment to Date*



*September 2005

Unither Pharmaceuticals

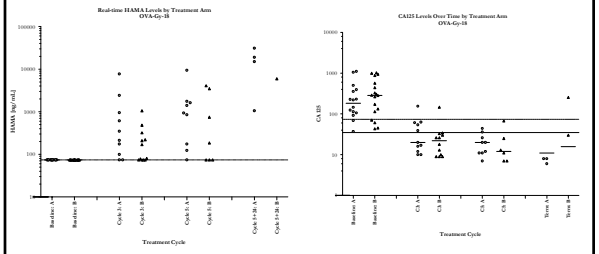
Protocol OVA-Gy-18
Piloting Front-Line Chemoimmunotherapy



Status: Enrolling at 7 centers
Preliminary Data 2006

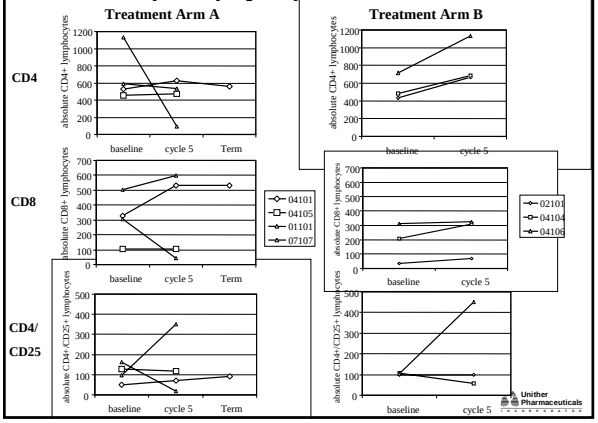
Unither Pharmaceuticals

OVA-Gy-18
HAMA & CA125 By Treatment Arm



Unither Pharmaceuticals

OVA-Gy-18 Lymphocyte Profiles Over Time



Unither Pharmaceuticals

Integrated Safety Experience:

Most Frequent Treatment-Emergent Adverse Events (OvaRex vs. Placebo)

MedRA System Organ Class (SOC)	Frequency (% of patients in each group)	
	OvaRex (N=360)	Placebo (N=196)
Gastrointestinal disorders	71.4%	78.6%
General disorders & administration site conditions	54.4%	63.3%
Muskuloskeletal &connective tissue disorders	51.1%	56.1%
Nervous system disorders	41.7%	56.6%
Infections and infestations	31.9%	36.2%
Respiratory, thoracic & mediastinal disorders	28.6%	39.8%
Skin and subcutaneous tissue disorders	20.6%	30.1%
Psychiatric disorders	21.1%	21.4%
Vascular disorders	18.9%	20.4%
Reproductive system and breast disorders	15.0%	15.8%

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.

Unither Pharmaceuticals

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

Unither Pharmaceuticals

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

Unither Pharmaceuticals

Acknowledgements

Research

Unither R&D
Joy Cieszynski
Birgit Schultes
L. Mary Smith

AltaRex / Virexx Research
Antione Noujaim
Hubert Eng

University of Pittsburgh
Theresa Whiteside
Lisa Butterfield

University of Maryland
Dean Mann

Clinical Investigators

James Bearden
Jonathan Berek
Douglas Blayney
Jeffrey Bloss
Patricia Braly
Sandra Brooks
Richard Buller
Linda Carson
Christina Chu
Robert Coleman
Mary J. Cunningham
Susan Davidson
Robert A. Burger
Johann S. de Bono
James Dolan
Robert Edwards
Michael Finan
Neil Finkler
Francine Foss
Holly Gallion
Agustin Garcia
Alan N. Gordon
Don Hall
Jean Hurteau
David Irwin
Ming-Shian Kao
Joseph Kelley
Stuart Lichtman
William McGuire
Michael Method
Boniface Ndubisi
James Orr
Giri Bula Patel
Jonathan Polikoff
Saul Rivkin
James Roberts
Harriet Smith
Peyton Taylor
Joan Walker
Steven Waggoner

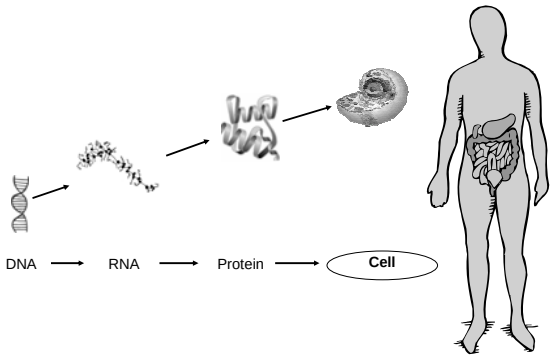
Additional Investigators (Canada, Europe, US)

Unither Pharmaceuticals

Individualized Cancer Care

New Technology
New Era

Molecular vs. Cell Based Determinants of Resistance



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

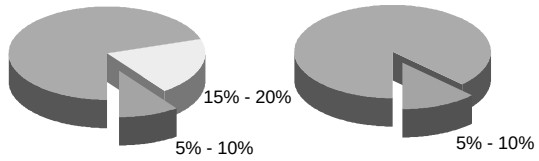
Ovarian Cancer: Hereditary Risk

Family History of Ovarian Cancer	Lifetime Risk
None	1.5%
1 first-degree relative	5%
2 first-degree relatives	7%
Hereditary ovarian cancer syndrome	40%
Known BRCA1 or BRCA2 mutation	35-65%

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 Ovarian Cancer

- ☐ Sporadic
- ☐ Family Cluster
- ☐ Hereditary

ASCO 1998

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

ASCO 1998

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

Prognosis: Breast Cancer as an Example of Genetic Prediction

- Current guidelines: chemo for most >1cm tumors
  - 80% will not recur with loco-regional RX and Tam
    - <20% recur within 10 yrs on Tam only
  - Many women are offered chemotherapy
    - Few will benefit
  - Guidelines assume all patients equally benefit
    - Some patients are under-treated
    - Many others are over-treated

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

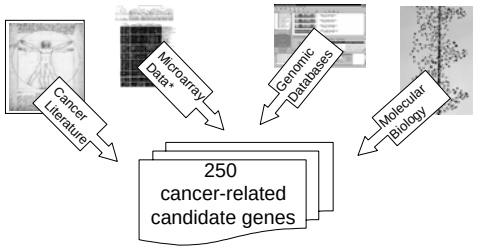
Outcome: NED 7 years

Clinical Course: ACT neoadjuvant therapy, progressive disease, mastectomy/radiation, Tam, Herceptin

Outcome: Local and distant recurrence at 6 months

Oncotype DX™ Technology: Candidate Gene Selection

From ~40,000 genes:



***Sources include: van't Veer et al, *Nature* 2002;415:530-6.**
Sorlie et al, *PNAS* 2001 98:10869-74.
Ramaswamy et al, *Nat Genet* 2003;33:49-54.
Gruvberger et al, *Cancer Res* 2001;61:5979-84.

Oncotype DX™ Technology: Final Gene Set

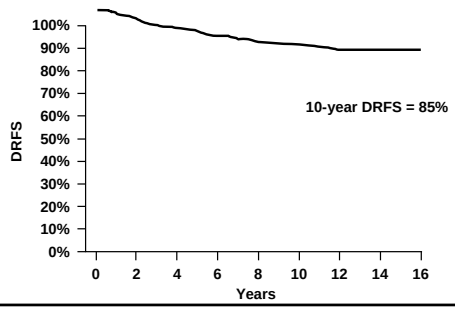
PROLIFERATION Ki-67 STK15 Survivin Cyclin B1 MYBL2	**HER2** GRB7 HER2	**ESTROGEN** ER PGR Bcl2 SCUBE2
	GSTM1	
INVASION Stromelysin 3 Cathepsin L2	CD68	**REFERENCE** Beta-actin GAPDH RPLPO GUS TFRC
	BAG1	

Oncotype DX™ Technology: Algorithm and Recurrence Score (RS)

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

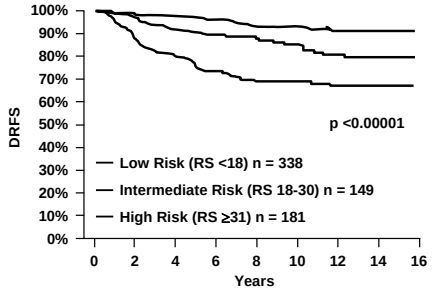
Oncotype DX™ Clinical Validation: Tam Only B-14 Results – DRFS

DRFS Over Time – All 668 Patients



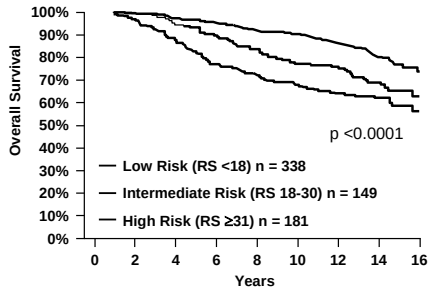
Oncotype DX™ Clinical Validation: B-14 Results – DRFS (cont)

DRFS for the Three RS Groups



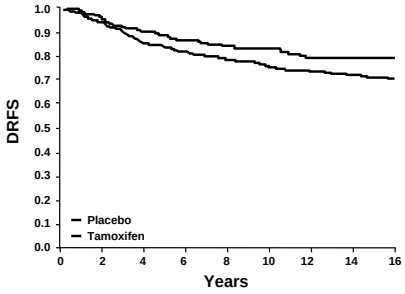
Oncotype DX™ Clinical Validation: B-14 Results – Overall Survival

OS for the Three RS Groups



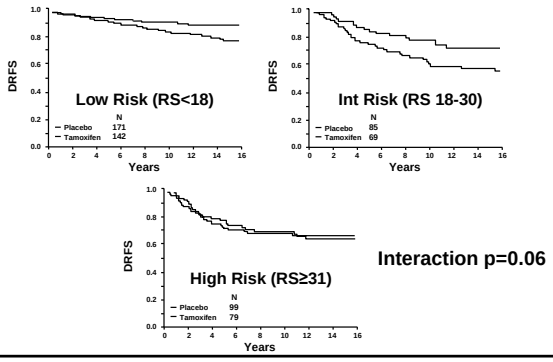
B-14 Overall Benefit of Tam

All Patients (N = 645)



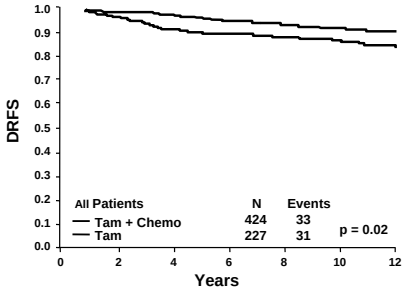
B-14 Benefit of Tam

By Recurrence Score Risk Category



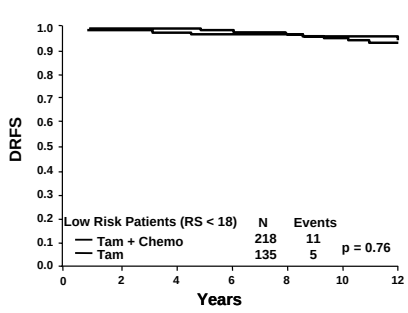
B-20 Results

Tam vs. Tam + Chemo – All 651 Pts



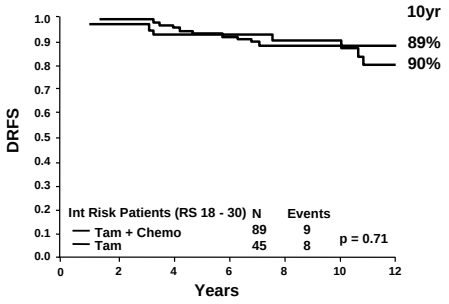
B-20 Results

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



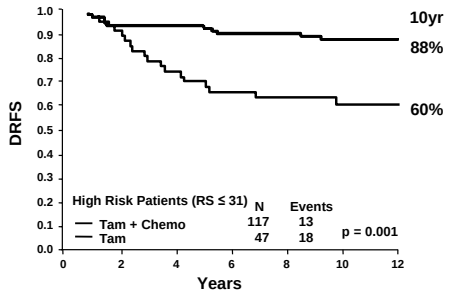
B-20 Results

Tam vs. Tam + Chemo – Int Risk (RS 18–30)

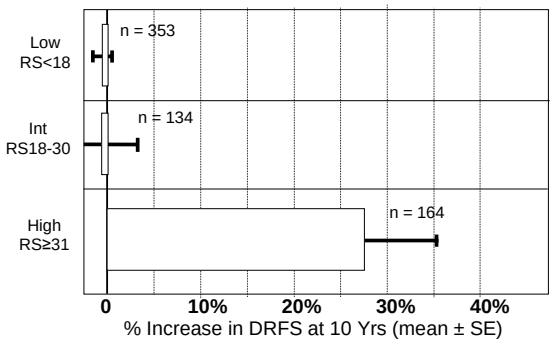


B-20 Results

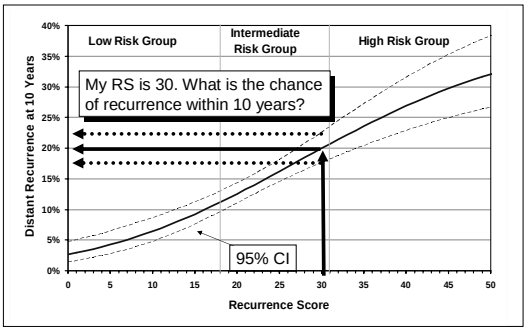
Tam vs. Tam + Chemo – High Risk (RS ≥ 31)



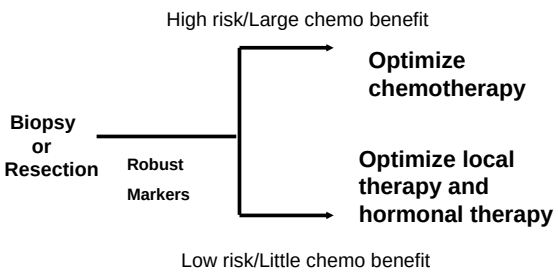
B-20: Absolute % Increase in DRFS at 10 Years



Oncotype DX™ Clinical Validation: RS as Continuous Predictor



Oncotype DX™



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 the 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

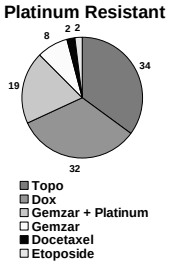
- Currently based on traditional prognostic factors:
  - Age
  - Stage
  - Grade
  - Cell Type
  - IHC

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



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.

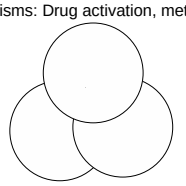
Limitations of Current Trial-and-Error Approach

- 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

All patients are not created equally

Patient

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



Drug
Delivery

Tumor

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

Individualized Selection of Therapy

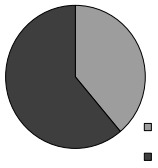
- Chemosensitivity and Resistance Assays
  - EDR assays
  - Chemosensitivity assays (ChemoFx®)
- Targeted Therapies
  - Histological markers – which patients are eligible for new therapies

Historical CSRAs vs. ChemoFx®

Historical Assays	Precision ChemoFx ®
Low Yield	High Assessability >90%
1-2 grams Required	35mg Required
Long Turn Around Time	10-14 days
Resistance Only	Sensitivity and Resistance
Non Standardized Procedures	Automatable, Reproducible

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



Taken from Great Debates in Gynecological Oncology, published by the Network for Oncology Communication and Research, 2003

Extreme Drug Resistance Assays

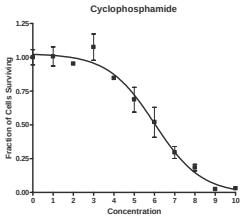
- Extreme Drug Resistance (EDR) Assays
  - Tumors placed in agar
  - Exposed to a single, very high concentration of drug
  - Measures proliferation
  - Only identifies drugs likely to <u>not</u> work
- Examples:
  - Oncotech EDR® Assay
  - Genzyme DRA™ Assay

Chemosensitivity Assays

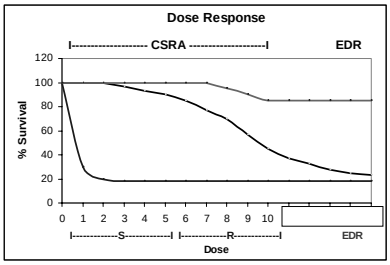
- Chemosensitivity (Response) Assays
  - Expose cells to increasing concentrations of chemotherapy
  - Measures cell death
  - Identifies drugs
    - won't work
    - are likely to work
- Examples:
  - ChemoFx® Assay by Precision Therapeutics
  - EVA™ Assay by Rational Therapeutics

ChemoResponse Calculated

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



CSRA vs. EDR: Individual Patient Results



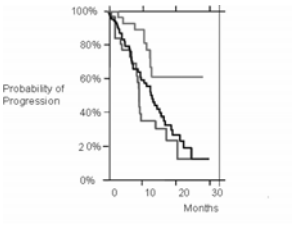
Chemotherapy Response Testing: Accuracy

- 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

Chemotherapy Response Testing: Accuracy

- In other words:
  - If the RR to a drug is 40%
    - Test sensitive and get the drug
      - Expected response is 57%
    - Test resistant and get the drug
      - Expected response is 9%

001: PFS According to ChemoFx® Assay Prediction of Response to Therapy Received: Exact Matches, n=135



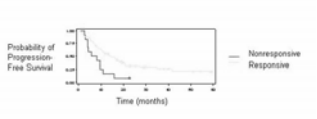
Resistant vs. Intermediate HR: 1.7 95% CI: 1.2 to 2.5

Resistant vs. Sensitive HR: 2.9 95% CI: 1.4 to 6.3 p<0.01

Median PFS: 9 mo for resistance, 14 mo for intermediate, not achieved in sensitive

ASCO Abstract 2004

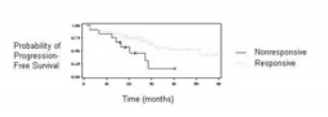
PFI According to ChemoFx® Assay Prediction of Response to Platinum Therapy (n=84)



	N	Median Progression	95% CI	P-value
Nonresponsive	12	6.95	4.1, 9.6	0.008
Responsive	72	14.26	10.6, 19.4	

Nonresponsive vs. Responsive **HR: 2.38** **95% CI: 1.23 to 4.76** **p=0.01**

Overall Survival According to ChemoFx® Assay Prediction of Response to Platinum Therapy (n=84)



	N	Median Survival (months)	95% CI	P-value
Nonresponsive	12	20.37	14.3, 28.7	0.02
Responsive	72	51.48	31.2, .	

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

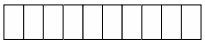
Predictive Value vs. Other Clinical Lab Tests

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

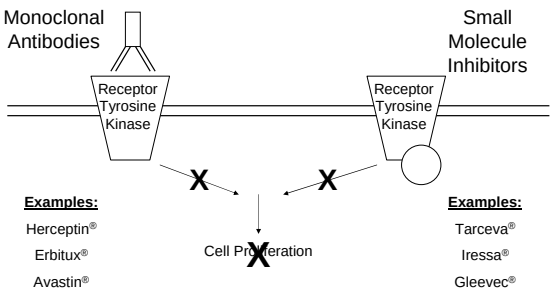
CSRAs vs. EDR Assays

- CSRAs provide information useful to most patients

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



Targeted Therapeutics



Targeted Therapeutics

Avastin® (bevacizumab)

Target: VEGF-R (vascular endothelial growth factor receptor)
Indication: Metastatic colorectal cancer

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

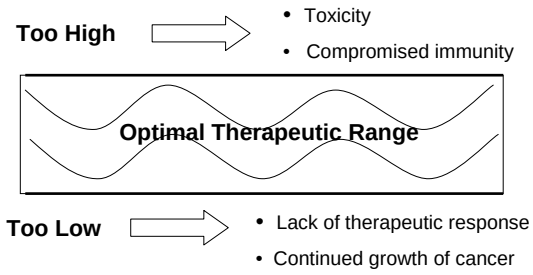
- Identification of high risk women
- Accurate prognosis
- Prediction of response to therapy
- Individualized dosing
  - Pharmacokinetics
    - Pharmacogenetics

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

* Pharmacokinetic

Finding the Optimal Therapeutic Range



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

CACAGCACATCG**C**G
CACAGCACATCG**T**G

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