Gynecologic Oncology Group
81st semi-Annual Meeting

Scientific Session Program
“The GOG Specimen Bank & Translational Research”

Thursday, July 15, 2010
4:00 pm - 6:00 pm
Sheraton Hotel Boston
Boston, MA
Faculty Disclosure Information

GOG Scientific Session “The GOG Specimen Bank and Translational Research”
July 15, 2010, Boston, MA

In compliance with ACCME regulations, the American College of Surgeons, as the accredited provider of this activity, must ensure that anyone in a position to control the content of the educational activity has disclosed all relevant financial relationships with any commercial interest. Members of the program committee were required to disclose all financial relationships and speakers were required to disclose any financial relationship as it pertains to the content of the presentations. ACS defines a “commercial interest” as any proprietary entity producing health care goods or services consumed by, or used on patients. The ACCME does not consider providers of clinical service directly to patients to be commercial interests. The ACS considers “relevant” financial relationships as financial transactions (in any amount) occurring within the past 12 months that may create a conflict of interest.

ACS is also required, through our joint sponsorship partners, to manage any reported conflict and eliminate the potential for bias during the activity. The program committee members (if applicable) and speakers were contacted and the conflicts listed below have been managed to our satisfaction. However, if you perceive a bias during a session, please report the circumstances on the session evaluation form.

Please note we have advised the speakers that it is their responsibility to disclose at the start of their presentation if they will be describing the use of a device, product, or drug that is not FDA approved or the off-label use of an approved device, product, or drug or unapproved usage.

The requirement for disclosure is not intended to imply any impropriety of such relationships, but simply to identify such relationships through full disclosure, and to allow the audience to form its own judgments regarding the presentation.

<table>
<thead>
<tr>
<th>NAME</th>
<th>NOTHING TO DISCLOSE</th>
<th>DISCLOSURE &lt;company &amp; role&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ronald Alvarez, MD (Planning Committee member)</td>
<td></td>
<td>GSK, Eli Lilly Ortho Biotech, Honorariums</td>
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<tr>
<td>Michael Birrer, MD (Planning Committee member)</td>
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<tr>
<td>Michael Cibull, MD (Planning Committee member)</td>
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<td>John Farley, MD (Planning Committee member)</td>
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<tr>
<td>Julie Gastier-Foster, PhD</td>
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<tr>
<td>Paul Goodfellow, MD</td>
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<tr>
<td>Neil Horowitz, MD (Planning Committee member)</td>
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<td>David Huntsman, MD</td>
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<td>Larry Maxwell, MD</td>
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<tr>
<td>Frederick Stehman, MD (Planning Committee member)</td>
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GOG Mission

The Gynecologic Oncology Group (GOG) is a non-profit organization (national) with the purpose of promoting excellence in the quality and integrity of clinical and basic scientific research in the field of Gynecologic malignancies. The Group (gynecologic oncologists, medical oncologists, pathologists, radiation oncologists, nurses, statisticians, basic scientists, quality of life experts, data managers and administrative personnel) is committed to maintaining the highest standards in clinical trials development, execution, analysis and distribution of results. Continuous evaluation of our processes is utilized in order to constantly improve the quality of patient care.

GOG CME Mission

The purpose of the GOG CME program is to provide and promote an infrastructure dedicated to enhancing the knowledge base of GOG meeting participants and guests centered on the development, execution, analysis and application of GOG-supported clinical trials. To that end, the CME Program engages in these discussions member researchers and invited clinicians committed to reducing the risk and improving outcomes for women at risk for or afflicted with a gynecologic malignancy.

GOG Meeting Registration Desk Hours

Thursday, July 15, 2010 - 7:00 am - 6:00 pm

Friday, July 16, 2010 - 7:00 am - 5:00 pm

Saturday, July 17, 2010 - 7:00 am - 4:00 pm
GOG SCIENTIFIC SESSION

“The GOG Specimen Bank & Translational Research”

Thursday, July 15, 2010
4:00pm - 6:00pm

Program Committee:
Michael L. Cibull, MD - Chair
Michael J. Birrer, MD, PhD - Co-Chair

Staff:
Zelika W. Compaore
Jill Reese
Michelle N. Small

Session Description:

The Gynecologic Oncology Group July 2010 Scientific Session is titled “The GOG Specimen Bank and Translational Research”, with noted Oncologists and Scientists serving as speakers. This 2-hour session addresses clinical questions among the various disciplines involved in the treatment of gynecologic cancers, with the objectives of illustrating the importance of banked specimens in supporting hypothesis-driven translational research in the cooperative setting. The targeted audiences are members and non-members of GOG research teams to include: Gynecologic Oncologists, Medical Oncologists, Pathologists and other MDs engaged in gynecologic oncology research and/or clinical practice; Oncology Nurses, Nurse-practitioners, Data Managers and other interested Allied Health professionals. The speakers will focus their presentations on the use of repository specimens for translational research and a question and answer session will conclude the session with audience participation.

Learning Objectives

◊ To illustrate the importance of banked specimens in supporting hypothesis-driven translational research in the cooperative group setting

◊ To demonstrate the wide range of studies that benefit from use of banked specimens

◊ To identify emerging technologies that extend the utility of banked specimens

GOG COMMITTEE ON EDUCATIONAL ACTIVITIES

| Ronald Alvarez, MD - Committee Chair | Frederick Stehman, MD – Co-Chair | Neil Horowitz, MD | John Farley, MD |
| University of Alabama School of Medicine | Indiana University School of Medicine | Dana Farber Partners Cancer Care | Walter Reed Army Hospital |
| Birmingham, AL | Indianapolis, IN | Massachusetts General Hospital | Washington, DC |
## Presentation Agenda
**Thursday, July 15, 2010**

<table>
<thead>
<tr>
<th>Time</th>
<th>Program Chairs</th>
<th>Session Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:00pm</td>
<td>Program Chairs</td>
<td>Welcome</td>
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<tr>
<td>4:05pm – 4:25pm</td>
<td>Paul Goodfellow, PhD</td>
<td>“Endometrial Cancer Tumor Studies: challenges and successes with bed side to bench research”</td>
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<tr>
<td></td>
<td>Washington University School of Medicine</td>
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<tr>
<td>4:30pm – 4:50pm</td>
<td>G. Larry Maxwell, M.D.</td>
<td>“Identification of Biomarkers Associated with Poor Prognosis in Endometrial Cancer”</td>
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<tr>
<td></td>
<td>Walter Reed Army Medical Center</td>
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<tr>
<td>4:55pm – 5:15pm</td>
<td>David Huntsman, M.D.</td>
<td>“High Resolution Genomics of Ovarian Cancer: Will it provide usable knowledge or more data?”</td>
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<td></td>
<td>University of British Columbia</td>
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<tr>
<td>5:20pm - 5:40pm</td>
<td>Julie Gastier-Foster, PhD</td>
<td>“Beyond the Freezers: Technologies and Services Available at the GOG Bank”</td>
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<td></td>
<td>Nationwide Children’s Hospital</td>
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<tr>
<td>5:40pm - 6:00pm</td>
<td>Program Chairs</td>
<td>Questions &amp; Answers / Wrap Up</td>
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</tbody>
</table>

For questions or comments about this CME activity, please contact Zelika W. Compaore, Manager, Education Programs and CME Compliance of The Gynecologic Oncology Group at: zcompaore@gog.org.
About The GOG Tissue Bank

The GOG Tissue Bank is focused on the long-term acquisition and storage of human tissues, cells, blood products and fluids to support and facilitate gynecologic cancer research.

- Opened June 1992
- Over 59,000 subjects enrolled
- Over 160,000 specimens stored
- Over 130,000 tissues distributed
- Over 250 institutions have contributed specimens
- 155 publications and abstracts
CME INFORMATION

The GOG has applied for Continuing Medical Education Credits (CMEs) for most of its meetings.

Important CME Information for Committee Members

CMEs can only be obtained by signing PINK sheets as you enter each session. They will be located at each entrance door. Sign-In Sheets cannot be signed after the session has finished. Committee members will not receive CMEs if they have only signed the committee sign-in sheets. They also must sign the CME sign-in sheets (pink).

Scientific Session CME Credits

A meeting evaluation form MUST be filled out in order to receive your CME credits. Evaluations will be available in the meeting room or at the CME evaluation desk. If you have pre-registered, the evaluations will be in your meeting packets. If you are registering onsite you can go to the CME registration desk to receive your certificate. All certificates for the Scientific Session will be validated after the completion of the course.

AMERICAN COLLEGE OF SURGEONS
ACCREDITATION/CME INFORMATION

Accreditation Statement
This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the American College of Surgeons and the Gynecologic Oncology Group. The American College of Surgeons is accredited by the ACCME to provide continuing medical education for physicians.

AMA PRA Category 1 Credits™
The American College of Surgeons designates this educational activity for a maximum of 2 AMA PRA Category 1 Credits™. Physicians should only claim credit commensurate with the extent of their participation in the activity.
Dr. Goodfellow is Professor of Surgery, Genetics and Obstetrics and Gynecology at Washington University School of Medicine and Siteman Cancer Center in St. Louis, MO. He received his PhD from Queens University in Kingston, Canada. Dr. Goodfellow completed a fellowship at Imperial Cancer Research Fund in London, England in the Laboratory of Human Molecular Genetics. He was an Assistant Professor at the University of British Columbia, Vancouver, Canada from 1988-1992. In 1992, he joined the faculty at Washington University School of Medicine where he plays important leadership roles in the Siteman Cancer Center, the Graduate Training Program and in Gynecologic Oncology Research. Dr. Goodfellow is an active member of the Gynecologic Oncology Group, a member of the NCI Gynecologic Cancer Steering Committee and the NIH College of Reviewers and has served on a variety of NIH study sections and special review panels. Dr. Goodfellow is the Director of the recently awarded SPORE in Endometrial Cancer at Washington University/ Siteman Cancer Center.

Dr. Goodfellow has long-standing focus on elucidating the causes and consequences of defective DNA mismatch repair in endometrial cancer and genomic approaches to improved detection and treatment of endometrial cancer.
Endometrial Cancer Studies
Challenges and Successes with Bedside to Bench Research

Paul J. Goodfellow, Ph.D.
No financial relationships to disclose

Patient Care
Novel approaches to prevention, detection and treatment of endometrial cancer

Genetics of tumor initiation and progression

Basic science investigations

Not so quick..this is not a simple process

Human Populations

Translation to patient care

\textit{in vitro} and \textit{in vivo} models

mouse cell line
From bedside to bench

The work requires partnerships and commitments that are unfamiliar to most investigators...a different kind of team science is needed. It also take resources (time, people and money...lots all of those and more) our current work world does not afford us.

Human Populations

- Women with definable phenotypes
- Tumors for molecular studies

Translation to patient care

in vitro and in vivo models

mouse cell line

Human Populations

- Large sample sizes required (GOG tissue bank)
- Logistical and bioinformatic/data management support systems (GOG and SDC)
- Commitment to best possible data and specimens...our only chance given the complexity of the disease and biologic system
Translation to patient care
- improved management of endometrial cancer probands and families with inherited cancer susceptibility
- targeted therapies

Why a Genetic Approach to Study of Endometrial Cancer?
- phenotypes that are likely to have definable genetic causes
  - histologic and biologic variation
- tumor tissues are accessible and available for investigation
  - cancers and precancerous lesions
- fraction of cases arise because of an underlying inherited cancer susceptibility
  - informative point of reference

DNA Mismatch Repair
(a key player in ensuring the overall integrity of the genome)
- recognition and repair of mutations
- apoptotic signaling
Epigenetic Change Associated with Loss of MMR in MSI-positive Endometrial Cancers

- Methylation of the MLH1 promoter (CpG island) seen in the majority of MSI-positive endometrial cancers and correlates with lack of MLH1 gene expression in sporadic tumors and tumor cell lines

Growing the Sample Size

- Big questions require bigger samples
- Follow-up studies on causes of defective DNA mismatch repair largely WUSM case but with help of GOG-136 extended to U Washington and Indiana University

Center is supported in part by National Cancer Institute Cancer Center Support Grant P30 CA016672. Some these samples were provided by Gynecologic Oncology Group Tissues Bank Grant U10 CA75169. Other investigators may have received specimens from the same subjects. This work was supported in part by National Cancer Institute Grant CA7774 (to P.J.G.).
Causes of Defective MMR: Most Cancers with MSI are Sporadic

441 consecutive endometrial cancer cases (median age 64.6 years, range 26-92)

MSI analysis (441 cases)
- MSI-H: 127 (28.8%)
- MSI-L: 10 (2.3%)
- MSS: 304 (68.9%)

MLH1 methylation analysis (177 cases)
- MLH1 unmethylated: 92 (52.2%)
- MLH1 methylated: 85 (47.8%)

MLH1 methylated: 127 (28.8%)
- MLH1 methylated: 0/10 (0%)
- MLH1 methylated: 92 (72.4%)
- MLH1 methylated: 1/40 (2.5%)

Other DMMR Genes Mutation in MSI-U Endometrial Cancers

0% unlikely

MSH6?

MSH6 and Cancer Risk circa 2003

- germline mutations rare
  - most have been identified in HNPCC kindreds or patients with familial colorectal cancer or early onset colorectal cancer
- appear to confer a particular risk for endometrial cancers
  - families with multiple members affected with endometrial cancer (N=3)
- studies focused primarily on cases in which MSH2 and MLH1 defects have been excluded looking under the wrong lamp post?
MSH6 Mutation & Methylation Analyses

- 100 endometrial cancer tumor DNAs evaluated
  - 30 MSI-high tumors lacking MLH1 methylation
- 7 germline mutations identified in patients
  - unselected for age of onset or family history
  - all endometrioid adenocarcinomas that are MSH6 and in which the MLH1 promoter is unmethylated
- median age at diagnosis 53.6 years
  - range 45 - 71 years
- 1.6% of all women with endometrial cancer have a MSH6 mutation

MSH6 Confer High Risk for HNPCC-Associated Cancer

- tumors not typically associated with DNA mismatch repair defects seen in mutation carriers
- hazard ratio estimates for Bethesda Criteria tumors*
  - females 15.7 (95% CI 6.6-37.3)
  - males 4.9 (95% CI 1.2-19.0)
- cumulative risk to age 70
  - male carriers
    - 29% (95% CI 10-28)
  - female carriers
    - 54% (95% CI 38-91)
  - female population (SEER): 6.3%

*Mark Jenkins, Center for Genetic Epidemiology, University of Melbourne, personal correspondence

Two more deleterious found in same cohort
• germline MSH6 mutations are “common” in endometrial cancer patients unselected for family or medical history

– MSH6 mutation testing is now standard of care for women suspected to have HNPCC and extended to at risk family members

• 26% risk for endometrial cancer by age 70 (compared with 44% for MSH2 and MLH1 cancers)

• 10% risk for colon cancer by age 70 (compared with 33% for MSH2 and MLH1 cancers)

…and risk for both increases to age 80

MSH6-Specific Clinical Recommendation
Translation to patient care

• improved management of endometrial cancer probands and families with inherited cancer susceptibility

Translation to patient care

• targeted therapies (anti-tyrosine kinase with specificity for FGFR2)

SPORE in Endometrial Cancer
Project 1: FGFR2 as a therapeutic target in endometrial cancer
**FGFR2 Mutations in Endometrial Cancer**

- Frequent activating FGFR2 mutations in endometrial carcinomas parallel germline mutations associated with craniosynostosis and skeletal dysplasia syndromes

- FGFR2 is a context-dependent oncogene (melanoma vs. endometrial cancer)

**Somatic Mutations in Endometrial Cancer and Germline Mutations in Craniosynostosis Syndromes**

- nature has already taught us a great deal about function of mutations

**FGFR2 Signaling in Endometrial Cancer**

- Mutations in FGFR2 in 12-16% of endometrial cancers
- Mutations in KRAS2 in ~15% of endometrial cancers
- Mutations in these two genes are mutually exclusive

- ERK is activated (phosphorylated) in >50% of endometrial cancers.
FGFR2 in Endometrial Cancer

- Is FGFR2 mutation associated with outcome? Is FGFR2 expression associated with outcome? Is FGFR2 activation associated with other tumor changes?
- Complex questions require large patient groups...with high quality tissues and high quality clinical data GOG-210 and GOG tissue bank.

R21 and SPORE funded studies linked to GOG210—it takes time, money, coordination and commitment

Work with CEM, GOG210 subcommittee, SDC and tissue bank...policies and procedures are spelled out --- read, reread, discuss and justify

Inhibition of FGFR2 Kills Endometrial Cancer Cells

Inhibition of Activated Fibroblast Growth Factor Receptor 2 in Endometrial Cancer Cells Induces Cell Death

FGFR2 Abrogation

Reversal of Phenotypes Associated with FGFR2 Mutation

RNA interference and inhibition of MEK-ERK signaling prevent abnormal skeletal phenotypes in a mouse model of craniostenosis

...mouse model points to clear therapeutic potential for inhibiting FGFR2
Human Populations
(people and the tumors they develop)

GOG recruitment of patients with recurrent or persistent endometrial carcinoma to a phase II trial
GOG 229 series

Translation to patient care

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Acknowledgments

Funding agencies including NCI, ACS, GCF and many others

People dedicated to research and care of women with endometrial cancers
Gynecologic oncologists Laboratory Scientists
Pathologists Statisticians Clinical Geneticist Pathologists
PhD students Fellows Technicians

Patients and their families

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Paul Goodfellow, PhD
COL Maxwell is the Chief of Gynecologic Oncology at Walter Reed and the Director of the Gynecologic Cancer Translational Research Center, a DOD-funded research consortium that is focused on development of personalized medicine for early detection, prevention and treatment of gynecologic cancer. Dr Maxwell graduated from the University Of North Carolina School Of Medicine with honors, performed his residency at William Beaumont Medical Center and completed his fellowship training at Duke University. He has served at Walter Reed Army Medical Center over the past decade and most of his research efforts have focused on the investigation of endometrial cancer.
Molecular Profiles Associated with Poor Prognosis in Endometrial Cancer

COL Larry Maxwell, M.D.
Director, GCTCOE
Chief, Division of Gynecologic Oncology
Walter Reed National Military Medical Center
15 July 2010

Financial Disclosure

No Relevant Financial Relationship to Disclose

Mortality Associated with Endometrial Cancer

- According to SEER, the mortality associated with uterine cancer has almost doubled over the past 20 years and has been associated with a steady increase in the incidence of disease

Uredo et al. AJOG 2008;198:218–26
Transcript Expression

Proteomic Profiling Using LC/MS-MS

Why is Improved Screening for Metastasis Needed?

- Avoid under-detection of occult cancer
- Avoid overtreatment of un-staged patients
- Identify patients with unrecognized micro-metastasis that are at risk for recurrence
- Avoid overtreatment of patients without metastasis
Multivariate Analysis of the Effects of Gyn-Onc Care on Outcome

<table>
<thead>
<tr>
<th>Variable</th>
<th>P-Value</th>
<th>HR</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Age</td>
<td>&lt;.0001</td>
<td>1.038</td>
<td>1.027 - 1.049</td>
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<tr>
<td>Co-morbidity Score</td>
<td>0.01</td>
<td>1.04</td>
<td>1.01 - 1.07</td>
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<tr>
<td>Number of Lymph nodes</td>
<td>0.0311</td>
<td>0.995</td>
<td>0.987 - 1.000</td>
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<td>Stage (II vs IV)</td>
<td>&lt;.0001</td>
<td>3.355</td>
<td>2.685 - 4.192</td>
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<td>Grade (1 vs 2)</td>
<td>&lt;.0001</td>
<td>1.792</td>
<td>1.462 - 2.197</td>
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<td>Histology</td>
<td>0.6364</td>
<td>0.951</td>
<td>0.731 - 1.272</td>
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<tr>
<td>Primary Surgery</td>
<td>&lt;.0001</td>
<td>0.461</td>
<td>0.35 - 0.606</td>
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<tr>
<td>Chemotherapy</td>
<td>0.6216</td>
<td>1.056</td>
<td>0.85 - 1.333</td>
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<tr>
<td>Radiotherapy</td>
<td>0.68</td>
<td>0.955</td>
<td>0.767 - 1.188</td>
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<tr>
<td>Lymphadenectomy</td>
<td>0.0169</td>
<td>0.736</td>
<td>0.572 - 0.946</td>
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<tr>
<td>Gyn-Onc</td>
<td>0.0242</td>
<td>0.78</td>
<td>0.628 - 0.968</td>
</tr>
</tbody>
</table>

Cost of Metastasis Screening

- Cost analysis shows an incremental cost-effectiveness ratio (ICER) of $8222 per year of life saved (YLS) compared to usual care.
- Testing remains cost-effective unless the rate of referral to a Gynecologic Oncologist for full staging exceeds 90%.
- Conclusions were insensitive to variations in test characteristics, costs of treatment and probability of adjuvant therapies.

Havrilesky et al: Gynecol Oncol 2009;112:526-30

Patients at Risk for Recurrence

Keys et al: Gynecol Oncol 2004;92:744-51
Transcript Expression Predictive of Recurrence

- Only 59% had staging which was not defined
- Stage II and IIA included
- No overall global gene expression differences between recurrent and non-recurrent cases
- Gene expression based score identified a significant difference in time to recurrence for high risk versus low-risk patients

Ferguson et al: Gynecol Oncol 2005;11:2252-7

Linear progression

- The tumor undergoes a series of genetic and epigenetic alterations selecting for autonomous clones that have metastatic potential
- Tumor cells are shed only late in primary tumor progression and therefore the metastasis is similar to the primary tumor, making detection of metastasis molecules in the primary tumor more robust

Klein CA: Nature 2009;9302-12

Parallel Progression

- Molecular alterations associated with metastasis may be present in some of the cells comprising the early primary tumor
- Independent accumulation of genetic and epigenetic alterations in the primary tumor compared with the metastasis
- Predicts greater disparity between the primary tumor and the metastatic lesion, that will make detection of metastasis molecules in the primary tumor more difficult

Klein CA: Nature 2009;9302-12
Prediction of Node Metastasis Using Gene Expression Analysis


Serum Based Proteomics Analysis of Metastasis: Node+ vs Node-

Serous + Endometrioid Endometrioid

Prediction of Retroperitoneal and Intraperitoneal Metastasis using Genomic and Proteomic Analysis of Primary Tumor

Oligonucleotide Array LC/MS-MS
Selection Criteria

- All cases of stage I disease meet GOG staging criteria (4 PN right and left, 1 PA node right and left, cytology)
- All stage I cases designated as being non-recurrent have at least a 3 year progression free interval
- Selected tissue specimens have <50% necrosis and >20% tumor

Pre-operative Model

- Objective: Identification of patient at risk for metastasis that need to be referred to a specialist for conventional staging (outcome is stage)
- Pre-analytical preparation of all specimens using LCM
- Discovery analysis
  - Transcript expression using Affy U133 plus 2.0
  - Proteomic expression using LC/MS-MS
- Ancillary analysis of primary vs metastasis using advanced stage cases

Selection and Quality Assurance: Metastasis

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Pass</th>
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<tr>
<td>Chart review</td>
<td>587</td>
<td>353</td>
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<tr>
<td>Histology review by Tissue Bank (&lt;50% necrosis and &gt;20% tumor)</td>
<td>260</td>
<td>177 (68%)</td>
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<tr>
<td>Histology review by our team</td>
<td>177</td>
<td>171</td>
</tr>
<tr>
<td>RNA check following LCM</td>
<td>171</td>
<td>139</td>
</tr>
<tr>
<td>QA check following array analysis</td>
<td>139</td>
<td>132 (50%)</td>
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</tbody>
</table>

- No correlation with institution of origin; surgical, pathological, or demographic data; % necrosis or % tumor; pre-analytical preparation method; RNA RIN; order of chips; GAPDH or actin ratio
QA of Transcript Expression Analysis

- Unsupervised analysis revealed 2 clusters that separated by quality of data
- Discovery set chosen on basis of better GAPDH and actin ratios after checking RNA degradation plots
- No correlation between quality of data and pre-analytical techniques, QA tissue parameters, analytical methodology, clinical, pathologic and other data to include institution of origin

Sample Sets: Metastasis Project

<table>
<thead>
<tr>
<th>Transcript Expression</th>
<th>Protein Expression</th>
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<tbody>
<tr>
<td>Discovery (65):</td>
<td>Discovery (75):</td>
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<tr>
<td>- Stage I: 29</td>
<td>- Stage I: 29</td>
</tr>
<tr>
<td>- Stage IIIC: 21</td>
<td>- Stage IIIC: 29</td>
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<tr>
<td>- Stage IV: 15</td>
<td>- Stage IV: 17</td>
</tr>
<tr>
<td>Validation (57):</td>
<td>Validation (67):</td>
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<tr>
<td>- Stage I: 28</td>
<td>- Stage I: 28</td>
</tr>
<tr>
<td>- Stage IIIC: 20</td>
<td>- Stage IIIC: 28</td>
</tr>
<tr>
<td>- Stage IV: 9</td>
<td>- Stage IV: 11</td>
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</tbody>
</table>

External Validation

- Validation using western blot, Taq-man and TMA with hysterectomy specimens
  - Frozen specimen from Duke
  - Paraffin specimens from GOG-210
- Validation using TMA or mass-spectrometry with EMB specimens
  - Retrospective collection from Magee
  - Prospective collection from the GYN-COE
External Validation using Protein Quantification

Post-operative Model

- Objective: identification of patients with shortened progression free survival that had micrometastatic disease undetected using conventional pathology techniques (outcome is recurrence)
- Pre-analytical preparation of all specimens using LCM
- Discovery analysis
  - Transcript expression using Affy U133 plus 2.0
  - Proteomic expression using LC/MS
- ncRNA analysis
- Validation using western blot, Taq-man, TMA, and or mass-spectrometry
  - Frozen and paraffin specimens from GOG-210
- Validation using specimens from GOG-249

Selection and Quality Assurance: Recurrence

- Chart review
  - Total: 408
  - Pass: 308
- Histology review by Tissue Bank (<40% necrosis and >20% tumor)
  - Total: 308
  - Pass: 199 (65%)
- Histology review by our team
- RNA check following LCM
- QA check following array analysis
  - pending receipt
  - pending receipt
Prognostic Models

Primary Non-recurrent Stage I versus Stage III/IV

Primary Non-recurrent Stage I vs. Primary Recurrent Stage I

Primary Stage III/IV versus Metastasis

- Initial model built with clinical factors and then proteomic classifiers added
- Differentially expressed transcripts with corresponding proteins not identified in discovery proteomics further evaluated
- Model validated internally using paraffin samples prior to external validation using an independent set
Dr. David Huntsman is Professor of Pathology and Laboratory Medicine at The University of British Columbia (UBC), a Staff Pathologist at the BC Cancer Agency (BCCA), and a Consulting Pathologist at the Vancouver General Hospital (VGH).

Dr. Huntsman attended medical school at Memorial University of Newfoundland from 1984–88 and after completing a rotating internship, practiced family medicine in Labrador for two years. Following his experience in family medicine, he entered and completed a pathology residency and trained in clinical molecular genetics at UBC and subsequently studied cancer genetics at Cambridge University, UK.

Dr. Huntsman is currently the Medical Director of the Centre for Translational and Applied Genomics (CTAG) at the BCCA, Interim Director of the BC multidisciplinary ovarian cancer research team (OvCaRe), co-Director of the Genetic Pathology Evaluation Centre (GPEC) at the Jack Bell Research Centre, VGH, and Associate Director of the Hereditary Cancer Program (HCP) at the BC Cancer Agency.

Dr. Huntsman has active research programs in hereditary gastric cancer as well as in the development of predictive and prognostic tissue based cancer biomarkers of a wide variety of tumor types. As collaboration is critical in his field, Dr. Huntsman happily leads and engages in a wide number of multidisciplinary research groups.
Disclaimers

- I have collaborated with and received research funds from Pfizer, Sanofi Aventis, Takeda and Novartis
- I have agreed to participate in an SAB for Pfizer

Outline

- Introduction to NGS
- Transcriptome profiling as a cost effective means to identify coding mutations
- The validation bottleneck
- What to sequence
- Clinical usage
Changing Paradigms for Cancer Care

- **Generic Cancer Care** (1990)
- **Stratified Cancer Care** (2010)
- **Individualized Cancer Care** (2020)

- Breast cancer
- Lymphoma
- Ovarian cancer
- Pancreatic cancer

Personalized Cancer Control

- The use of tumour characteristics to determine optimal PERSONALIZED therapeutic choices: what a tumour is becomes the major determinant of care
- The development and application of surrogate markers for patient response
- The use of germline genetics to stratify cancer risk in populations
- The use of germline genetics to determine dosing and predict adverse reactions

The Move Towards Individualized Care

- 1) One gene at a time biological approach: yielded Her2, ER, PR, BRCA1 and BRCA2 etc
- 2) Looking at everything at once
Time to Throw Away the microscope!

Molecular classification of breast cancers in the clinic

- Oncotype Dx and other commercial assays exist
- Why no usable molecular classifiers for ovarian cancer?

What is really needed

- Base pair resolution view of germline and tumour genome
- Ability to study intratumoural heterogeneity
- Ability to study the emergence of drug resistance
Recent studies shaping cancer genomics

- Wood et al. 2007 (single base substitution and small in/del)
  - Examined ~21,000 transcripts (~18,000 genes)
  - 11 colorectal and 11 breast tumor samples with 2 matched normal controls
  - Non-synonymous mutations in 1,718 (9.4%) genes in at least 1 of the colon or breast tumors examined
  - 92.7% single base substitutions; 7.3% in/del
  - 280 potential driver mutations with ~15 in any single tumor
- Jones et al. 2008
  - 24 Pancreatic tumors
  - 1,562 somatic non-synonymous mutations

Lessons learned?

- Every tumor is different.
  - Heterogeneity
  - Driver versus passenger mutations
  - Pathway interactions
- Every cancer patient is different.
  - Diverse genetic backgrounds

Analysis of a small numbers of tumor/normal pairs will generate insufficient data.
- ICGC goal is 500 tumor/normal pairs for each cancer type.

Long capillary sequence reads
New generation DNA sequencers

1 instrument: 3,000,000,000 bp / day
- reads are short (eg 75 bp)
- designed for re-sequencing

~3,400 instruments: 3,000,000,000 bp / day
- reads are long (eg >750 bp)
- designed for de novo sequencing

Next generation sequencing

1 Flowcell

~300 million sequences at 75bp
>25 billion nucleotides

Repeat cycle ~50-100 times

1 week

Base calling

The sequence of a cluster is deduced by analysis of sequential images
Unique sequence alignments and read length

Vistas Opened Through New Sequencing Technologies

- Causes of cancer susceptibility
- New diagnostics and targets for treatment
- Mechanisms of drug resistance
- Clinical importance of intratumoral heterogeneity
- New insights in cancer biology such as RNA editing

Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing

Human cancer often harbors genome rearrangements, some of which are implicated in cancer development. However, conventional methods for identifying rearrangements are laborious and time-consuming. To address these limitations, we developed a genome-wide paired-end sequencing strategy. By analyzing paired-end reads, we were able to identify rearrangements with high sensitivity and specificity. Our approach allowed us to detect rearrangements that are rare or small, which are often missed by other methods. These findings have important implications for our understanding of cancer biology and have potential applications in personalized medicine.

Nature Genetics 2008
Lung cancer genomes are complex

Lung cancer genomes are even more complex

Mutational evolution in a breast cancer

What mutations were present in the metastatic tumour?
How many new mutations/aberrations arose?

NGS sequences a subset of the population of alleles in the sample

Total DNA/RNA extraction

Library construction separates DNA templates and each one is sequenced independently

Mutational evolution of a lobular breast cancer

21 of the genes with mutations had not been described in cancer before

Only 2 of the genes were mutated in 192 other breast cancers

Only 11 mutations were present in the primary tumour

None of these 32 genes are in common with the CAH genes surveyed from ER- breast cancers

Transcriptomes

RNA → poly-A+ → Random-primed cDNA → Fragment and sequence.

- mRNA
- rRNA
- miRNA
- tRNA
- snRNA
- ...
Why Transcriptome sequencing?

- Transcriptome is much smaller than genome
- Measure / detect:
  - Alternative transcription / splicing (Sultan et al, 2008).
  - RNA edits (Shah et al, 2009b).
  - Fusion transcripts (Maher et al, 2009).
  - “Point” mutations (Morin et al, 2008; Shah et al., 2009a,b).
Ovarian cancer control

- No screening test
- No biomarkers routinely used for management
- Subtype specific management accepted as a concept by a portion of the community
- Major advances of past ten years are the identification of women at high risk through BRCA mutations, the development of PARP inhibitors as a treatment of BRCA deficient cancers and the identification of credible precursor from the study of fallopian tubes from prophylactic surgeries

If the subtypes are distinct diseases, What this means for ovarian cancer genomics projects?

- Each subtype must be considered to be a distinct entity worthy of study
- Data from one subtype can be played against other subtypes
Her-2 amplification as a predictive biomarker for mucinous carcinomas
Endometrioid carcinomas

- Low grade
- Chromosomally stable
- Endometriosis associated
- Wnt and PI3K driven

Concordance of mutational and expression signatures by next gen sequencing with IHC in endometrioid ovarian cancer
Three flavors of cancer: an approach to cancer genomics

- High grade cancers
- High grade serous cancer
- Pathognomonic mutations unlikely

- Moderate grade cancers
- Clear cell cancer
- Mutations in specific pathways that will be important in other cancers

- Unusual tumours with pathognomonic features
- Granulosa cell tumor of the ovary
- Pathognomonic mutations
Type 3 Cancers; Example - Granulosa cell tumours

16 Cancers: What to study?

See Figure 3 (main text)

FOXL2 mutation in all 4 granulosa cell tumors of the ovary

FOXL2 essential for maintaining female state of GC's, Uhlemhaut et al Cell 2009

The FOXL2 mutation in GCT's
- Present in 86/89 (97%) of adult type GCT's
- Not present in 1050 unrelated cancers
- This is the first diagnostic for GCT's
- External validation Kim et al J Path 2010-53/56 aGCT mut +, 0/1290 other tumours carry mutation
Putative single nucleotide variants from RNA seq databases

- Artifacts: not in tumour genome or transcriptome
- Novel coding germline SNP’s: in tumour genome, transcriptome and normal germline DNA
- Somatic mutations: in tumour genome and transcriptome but not in germline DNA
- RNA edits: in transcriptome but not in neither the tumour nor germline DNA

Comparison of the transcriptome and genome reveals widespread RNA editing

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Description</th>
<th>Edits</th>
<th>Non-synonymous edits</th>
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</thead>
<tbody>
<tr>
<td>EIF2AK2</td>
<td>eukaryotic translation initiation factor 2-alpha kinase 2</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>AKAP9</td>
<td>A kinase (PKA) anchor protein (syntaphilin) 9</td>
<td>25</td>
<td>0</td>
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<tr>
<td>COG3</td>
<td>COG3 component of oligomeric gpg complex 1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ICK</td>
<td>intestinal cell Wyk-lik kinase</td>
<td>2</td>
<td>0</td>
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<tr>
<td>EIF2R</td>
<td>eukaryotic elongation factor-2 kinase</td>
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<td>0</td>
</tr>
<tr>
<td>BMPR2</td>
<td>BMP receptor 2</td>
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<td>0</td>
</tr>
<tr>
<td>FAM38A</td>
<td>Family 38 homology sequence A</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>SRP9</td>
<td>Signal sequence recognition peptide</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

ADAR (adenosine deaminase) – one of the top 5% of genes expressed

RNA editing in SRP9 and COG3

Sanger amplicon sequencing in tumour RNA and tumour DNA in COG3 and SRP9 confirms non-synonymous coding RNA edits
Chromosomal instability and fusion proteins are key features in ovarian cancer. Fusion Discovery With RNA-Seq involves:

1. Clustering of paired-end reads based on fragment probability distribution.
2. Searching for reads split by breakpoint.

Thus, six confirmed fusions and isoforms are identified.
Validation of putative mutations

- For singular events: taqman, mass spec etc
- For mutations across genes or gene families: Sanger sequencing, NGS of amplicons, or exon capture
For singular events: Taqman, mass spec etc
For mutations across genes or gene families: Sanger sequencing, NGS of amplicons, or exon capture
Alternative: start with more complete dataset

Experimental design for somatic mutation profiling

- Exome tumour
- Exome normal
- RNASeq tumour
- Integration

Somatic mutations (expressed and not expressed), gene expression, gene fusions, RNA edits, allelic expression, loss of heterozygosity
Single genomes a dozen and counting - what should we sequence next?

Could Tumor and/or Germline Sequencing Become Part of Standard Cancer Care: Challenges

- Cost of sequencing (diminishing)
- Cost of data storage (mundane)
- Development of methods for identification, validation and reporting of all clinically relevant findings
- Delineation of responsibilities and obligations
- Privacy and other ethical issues
- Need to prepare the broader clinical community for individualized genome driven care
On the cost of human genome sequencing

“I have my genome on my IPOD”

NGS of tumours in the cancer clinic

- The identification and reporting of common genomic features would be unlike other laboratory tests only in scale
- However, no identifiable group of clinicians or researchers is ready to deal with the residual information – CFTR mutations in cancer genomes etc
A new lens on cancer will lead to improved outcomes

- The redefined mutational landscape will yield new diagnostics, and targets for new therapies in cancer
- Ultimately this will lead to better outcomes for patients

Thanks

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Julie Gastier - Foster, PhD
Director, Cytogenetics / Molecular Genetics Laboratory
Nationwide Children’s Hospital

Associate Professor, Clinical
Department of Pathology
The Ohio State University College of Medicine

Dr. Gastier-Foster serves as the Director for the Cytogenetics / Molecular Genetics Laboratory at Nationwide Children’s Hospital. She is also the Scientific Director of the Children’s Oncology Group (COG) Acute Lymphoblastic Leukemia Molecular Reference Laboratory, the COG Neuroblastoma Reference Laboratory, and the COG Wilms Tumor Reference Laboratory. Dr. Gastier-Foster serves as an Assistant Medical Director of the Biopathology Center (including the Gynecologic Oncology Group tumor bank), overseeing the nucleic acid extraction core. She is also the Principal Investigator of the Nationwide Children’s Hospital Biospecimen Core Resource of The Cancer Genome Atlas project.

Dr. Gastier-Foster received her BS in Biotechnology from the Rochester Institute of Technology in Rochester, NY, and her PhD from Harvard Medical School in Boston, MA. She completed her Medical Genetics Postdoctoral Fellowship at Stanford University School of Medicine in Stanford, CA. She is board certified in Clinical Molecular Genetics and Clinical Cytogenetics. Dr. Gastier-Foster has more than 18 years of molecular genetics experience and 12 years of cytogenetics experience, including more than 8 years experience in processing and testing tumor specimens. Dr. Gastier-Foster’s primary areas of interests are molecular analysis of cytogenetic rearrangements and genetic analysis of human tumors.
Beyond the Freezers: Technologies and Services Available at the GOG Bank

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To understand the nucleic acid isolation, virtual microscopy, and tissue microarray (TMA) technology available in the GOG bank

To consider testing or development that could be centralized at the GOG bank

Conflict of Interest

Conflicts to disclose: None

Objectives

• To understand the nucleic acid isolation, virtual microscopy, and tissue microarray (TMA) technology available in the GOG bank

• To consider testing or development that could be centralized at the GOG bank
Biopathology Center

- Gynecologic Oncology Group (GOG) Tissue Bank
- Children's Oncology Group (COG) Tissue Bank
- Pediatric Cooperative Human Tissue Network (pCHTN)
- Childhood Cancer Survivor Study (CCSS)
- Cancer Therapy Evaluation Program (CTEP)
- The Cancer Genome Atlas (TCGA)

Biopathology Center – Scope of Services

- Receiving / Banking / Distribution
- Core Morphology
- Pathology Review
- Virtual Microscopy
- Nucleic Acid Isolations
- Clinical Reference Laboratory
- Informatics

BPC – Receiving / Banking / Distribution

Specimens received: fresh, frozen, FFPE, formalin fixed, blood, blood products, bone marrow, urine
- GOG: 50 protocols (open and closed)
BPC – Banking and Storage

- Long-term mechanical (-20°C, -80°C) and liquid nitrogen cryostorage
- Rees freezer monitoring system

GOG Specimens Distributed

BPC – Core Morphology

- CAP-certified histology lab
- Embed tissue (paraffin, OCT)
- Paraffin and frozen tissue sectioning
- Immunohistochemical staining
- Laser-capture microdissection
- Tissue microarrays (TMA)
GOG TMAs

- Late Stage Ovarian
- Endometrial Carcinoma
- Ovarian Disease
- Progression
- Ovarian Stage
- Ovarian Histologic Subtype

BPC – Pathology Review

- Coordinate distribution of slides for rapid pathology review
- Determine % tumor / necrosis for clinical testing or nucleic acid isolations
- QA of tissue sections served to investigators
- Identification of regions of paraffin-embedded samples for TMA creation

BPC - Virtual Microscopy

Virtual Imaging Pilot EndeavoR

- Technique of digitizing microscope slides
- Access from anywhere
- Image analysis
- Slide conferencing
- Digital archive

Julie Gastier-Foster, PhD
BPC – Nucleic Acid Isolations

DNA and RNA extractions with quality control
- genomic DNA routinely for most GOG Phase II and Phase III studies
- work with investigators to choose optimal methods and optimal quality control for downstream assays

BPC – Clinical Reference Laboratory

- Clinical testing of protocol specimens in CAP/CLIA-certified lab
- Conversion of research findings to robust clinical assays

COG Neuroblastoma Risk Groups

Age
Tumor Stage
Shimada Path Review
MYCN Amplification
DNA Index
LOH 1p36, LOH 11q23

Low Risk
Intermediate Risk
High Risk
Neuroblastoma Risk Factors

- Age
- Stage
- MYCN
- Shimada
- DNA Index

MYCN Oncogene Amplification

Unbalanced LOH 11q23

Attiyeh, et al. 2006
BPC – Clinical Reference Laboratory

Methods used in clinical trials

- FISH
- RT-PCR
- DNA ploidy analysis
- LOH (genotype analysis)
- Expression studies
- Copy number analysis
- Targeted sequence analysis

BPC – Informatics

- Building and implementing comprehensive specimen tracking system (receiving, storage and distribution)
- Integration of disparate databases (data harmonization)
- Custom informatics application development

Need for additional biomarkers

![Graph showing probability of survival vs. years]

P=0.0007

Julie Gastier-Foster, PhD
The success of the Biopathology Center including the GOG bank is dependent on a large group of people with various expertise working together to achieve the scientific and research aims of the clinical trials of the cooperative groups.

Clinical testing has become a critical aspect of supporting clinical trials as research findings are converted into assays used for patient care decisions.
Scientific Session
Thursday, July 15, 2010
4 pm – 6 pm
Grand Ballroom/Liberty

The Gynecologic Oncology Group wishes to thank AMGEN ONCOLOGY for their grant support for this educational activity.
Save the Dates for these upcoming GOG Semi-Annual Meetings!

January 28-30, 2011
Manchester Grand Hyatt
San Diego, CA
(Symposium - January 27)

July 15-17, 2011
Philadelphia Marriott Downtown Hotel
Philadelphia, PA
(Symposium - July 14)

January 27-29, 2012
Manchester Grand Hyatt
San Diego, CA
(Symposium - January 26)

July 27-29, 2012
Sheraton Hotel
Boston, MA
(Symposium - July 26)

January 25-27, 2013
Manchester Grand Hyatt
San Diego, CA
(Symposium - January 24)

July 19-21, 2013
Marriott River Center
San Antonio, TX
(Symposium - July 18)

January 24-26, 2014
Manchester Grand Hyatt
San Diego, CA
(Symposium - January 23)

July 18-20, 2014
Hyatt Regency Hotel
Chicago, IL
(Symposium July 17)

January 23-25, 2015
Manchester Grand Hyatt
San Diego, CA
(Symposium - January 22)