10th International Symposium
Advanced Ovarian Cancer Optimal Therapy Update
Valencia 3/6/2015

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Director Gynecologic Medical Oncology
Massachusetts General Hospital
Conflict of Interest Disclosure

Michael J. Birrer

GSK, Bipar, Genentech
Advisory Board
Ovarian Cancer
The Clinical Problem

• 250,000 women diagnosed yearly worldwide
• 75% patients present with advanced stage disease.
• 80% respond to chemotherapy.
• Vast majority of patients relapse and eventually develop drug resistant disease.
• Minimal increase in overall survival over last 30 years.
• 140,000 deaths yearly.
• Highest case fatality rate for gynecologic cancers in the world
• All ovarian cancers treated with surgery/chemotherapy
Ovarian Cancers

- Serous: A disease of genomic instability.
- Mucinous: A disease of aberrant Ras pathway signaling
- Endometrioid: Multiple diseases. True endometrioid a disease of aberrant PTEN, PI-3K, AKT signaling
- Clear Cell: A disease of ARID1A
CLEAR CELL CANCER

- OVARIAN
- ENDOMETRIAL
- RENAL

Zorn et. al. Clinical Cancer Research 2005
Clear Cell Ovarian Cancer has unique targetable pathways

Cell Migration

Cell Cycle Progression

HIF1alpha degradation

Glycolysis

Angiogenesis
Clear Cell Specific Trials

GOG0254 A Phase II Evaluation of SU11248 (Sunitinib Malate) in the Treatment of Persistent of Recurrent Clear Cell Ovarian Carcinoma (John K Chan)

GOG0268 A Phase II Evaluation of Temsirolimus (CCI-779) (NCI Supplied Agent: 683864, IND #61010) in Combination with Carboplatin and Paclitaxel followed by Temsirolimus Consolidation as First-line Therapy in the Treatment of Clear Cell Carcinoma of the Ovary. (John H Farley)
Papillary Serous Ovarian Cancer

Low-Malignant Potential (LMP)

(40x Magnification)

Invasive Carcinoma

(40x Magnification)
Ovarian Cancer

Papillary Serous Ovarian Tumors

Normal Cells

P53-

P53+

High Grade

LMP/Low Grade

B-raf, ras

Bonome et al. Cancer Research 2005
A PHASE II TRIAL OF AZD6244 IN WOMEN WITH RECURRENT LOW-GRADE SEROUS CARCINOMA OF THE Ovary OR PERITONEUM: A Gynecologic Oncology Group Study

Farley J, et al Lancet Oncol. 2013 Feb;14
**MAPK pathway Inhibition is effective for Low grade tumors**

<table>
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<th>% 1 prior ChTx</th>
<th>% &gt; 3 prior ChTx</th>
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- exhibits considerable activity with minimal toxicity in recurrent low-grade serous tumors.
  - The 15% RR is 5X that observed for cytotoxic chemotherapy in the setting of recurrent low-grade serous tumors.
- These results warrant further evaluation of inhibitors of the MAPK pathway in low-grade serous ovarian cancers

1. Gershenson, Gynecol Oncol 2009;114:48
What about High Grade Cancers?

Early versus Late Stage Disease
Early Stage High Grade Ovarian Cancer

• Standard of care for early stage high grade epithelial ovarian cancer (ESHGOC) is the same as its advanced counterpart.

• 10-20% will have a recurrence despite complete resection and adjuvant chemotherapy;

• 50-60% of patients with ESHGOC will not suffer recurrent disease even in the absence of adjuvant chemotherapy;

• Many women over treated

• 2 approaches so far:
  – Clinical biomarkers algorithms
  – Gene/proteins characterizations

• Limitations:
  – Small datasets
  – Not prospectively validated
Early Stage High Grade Ovarian Cancer

• TCGA provided a better understanding of the genomic and transcriptomic alterations in advanced stage ovarian cancer.

• Only 24 out of 489 fresh frozen samples from primary surgery were early stages (IIA) and stage I were intentionally excluded.

• Biomarkers of recurrence and druggable pathways are lacking in OC and still represent the most important unmet need in science community.
Aim and impact

Know* the whole genomic characterization and gene expression abnormalities in ESHGOC can lead to:

– Identify a **prognostic-recurrence signature** that would stratify patients that could benefit from chemotherapy;
– Provide new opportunities to identify **early genomic changes** in ovarian cancer, **pathway activations**,;
– Offer insight into **novel therapeutic targets** and potentially **early detection biomarkers**;

– Potentially detect tumors in an early and curable stage, and tailor personalized treatment;

*: 2012P001330 protocol approved from Partners Healthcare IRB, latest update July 8th 2014
Methods (2): Macrodissection

- Creysl violet staining to facilitate macro-dissection
- Dissected samples to provide 80% tumor tissue;
- Stored also stromal tissue for tumor-related stroma analysis;

Example 1:

118-MGH (S0054782-B)

- **Tumor >90%**
- **Stroma <10% Tumor cells**
Methods: DNA CNV and ncRNA array based expression analysis(7)

• **DNA CNV Analysis** (IlluminaHumanOmniExpress-FFPE BeadChip system)
  - To characterize the copy number gain and loss for early stage high grade ovarian cancer

• **Non-coding RNA Expression Analysis** (Affymetrix microarrays)
  - Identification of small ncRNA signature predictive of tumor recurrence in early stage ovarian cancer

Integration analysis of small ncRNA signature with mRNA and CNV data to identify key small ncRNAs driving the recurrence of early stage ovarian cancer
Methods: RNA Seq, analysis (6)

(1) Training set, analysis, signature assessment,

(2) Validation set
• Planned to compare fresh frozen to FFPE samples to have information on RNA and DNA yield and signature;
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*N*: to be confirmed
Acknowledgments

• **Birrer Laboratory:**
  – Wei Wei, PhD
  – Lorenzo Ceppi, MD
  – Gayatry Mohapatra, PhD
  – Young Jeong Na, MD, PhD
  – Tsun Yee Tsang, PhD
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  – Giulia Fulci, PhD

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  – Svitlana Tyekucheva, PhD
  – Victoria Wang, PhD

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  – Michaela Bowden, PhD

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  – Sami Amr, PhD, FACMG

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  – DOD grant #OC110628, Birrer PI

• **Ovarian Cancer Research Fund fellowship program**
What about High Grade Cancers?

Early versus Late Stage Disease
TCGA Analysis of HGSOC

Lots of CNV and low mutation rate

Methylation, expression microRNA subsets?
Can we exploit the DNA repair deficiency and genomic instability?

HRD and Parp Inhibitors
PARP inhibition and tumor-selective synthetic lethality

DNA damage (SSBs)

DNA replication (accumulation of DNA DSBs)

PARP inhibition

Normal cell with functional HR pathway

HR-mediated DNA repair

Cell survival

Tumor-selective cytotoxicity

HR-deficient tumor cell (e.g. BRCA 1/2⁻/⁻)

No HR-mediated DNA repair

Cell death

DSB, double-strand break; HR, homologous recombination
SSB, single-strand break

Best % change from baseline in target lesions

Olaparib 400 mg bid cohort

*Platinum-sensitive patients. Figure includes 3 unconfirmed responses
Summary of Cancer-Associated Mutations: GOG 218 and GOG 262

- BRCA1: 44.2% (N = 117)
- BRCA2: 26.0% (N = 69)
- BRIP1: 7.5% (N = 20)
- PALB2: 3.4% (N = 69)
- ATM: 3.0% (N = 20)
- NBN: 2.6% (N = 20)
- RAD51C: 2.3% (N = 20)
- RAD51D: 2.6% (N = 20)
- CHEK2: 2.6% (N = 20)
- SLX4: 0.4% (N = 20)
- FAM175A: 0.4% (N = 20)
- TP53: 0.8% (N = 20)
- XRCC2: 0.4% (N = 20)
- LYNCH: 1.9% (N = 20)
- ATR: 0.8% (N = 20)
- BARD1: 0.8% (N = 20)

Total mutations: 265 in 258 women
In a tumor with genomic instability can we identify specific therapeutic targets?
Meta-analysis overview

**Literature review**

**Prognostic models**
- 101 candidate papers
- Five review papers

**Inclusion Criteria**
- Training sample size > 40
- Focus on late-stage serous
- Multivariate model
- Continuous risk score
- Claims to predict survival
- Possible to reproduce model

14 prediction models implemented
- 100 pages documentation
- *survHD* Bioconductor package

**Database of curated gene expression**
- Standardized clinical annotation and gene ID
- 23 studies, 2,908 samples

**Inclusion Criteria**
- Sample size > 40
- Primary tumors
- Overall survival available
- Events (deaths) > 15
- Late stage, high grade tumors
- Serous subtype

10 datasets, 1455 samples
- *curatedOvarianData* Bioconductor package
Assessment of prognostic signatures

\[ C = 0.5 \text{ expectation for random prediction} \]

\[ C = 1 \text{ if the exact order of all deaths is predicted} \]

\[ C-\text{Index} = \Pr(g(Z_1) > g(Z_2) \mid T_2 > T_1) \]

\[ T_1, T_2 = \text{times to death of two patients} \]

\[ g(Z_1), g(Z_2) = \text{predicted risk scores} \]
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14 prognostic signatures

10 microarray datasets

Validation Statistics for 14 Models in 10 Datasets

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Kaplan-Meier estimate

Survival

Time

0.0 0.2 0.4 0.6 0.8 1.0
0 20 40 60 80 100
Assessment of prognostic signatures

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Assessment of prognostic signatures

\[
C\text{-Index} = \Pr(g(Z_1) > g(Z_2) \mid T_2 > T_1)
\]

\(T_1, T_2\) = times to death of two patients
\(g(Z_1), g(Z_2)\) = predicted risk scores

C=0.5 expectation for random prediction
C=1 if the exact order of all deaths is predicted

<table>
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14 prognostic signatures

10 microarray datasets

Forest plot

Study

C-Index

0.4 0.5 0.6 0.7 0.8
Assessment of prognostic signatures

C-Index = Pr(g(Z₁)>g(Z₂) | T₂>T₁)

C=0.5 expectation for random prediction
C=1 if the exact order of all deaths is predicted

10 microarray datasets

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T₁, T₂ = times to death of two patients
g(Z₁), g(Z₂) = predicted risk scores

Forest plot
Assessment of prognostic models


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10 microarray datasets
Assessment of prognostic models

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Assessment of prognostic models

Conclusions:

- Most models make better predictions than random
Assessment of prognostic models

Conclusions:

- Most models make better predictions than random
- Large, consortium studies performed best
Assessment of prognostic models

Conclusions:

- Most models make better predictions than random
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- Validation datasets can be biased
Assessment of prognostic models

Conclusions:

- Most models make better predictions than random
- Large, consortium studies performed best
- Validation datasets can be biased
- None of these models are ready for the clinic
Application of Large Database for the Generation of Clinically Relevant Signatures

Patient Survival
A) Overview

Database of curated gene expression (23 studies) → 13 studies with survival information and sufficient sample sizes → 6 studies meeting training criteria, use these to develop a gene signature

Validate the meta-analysis method in training datasets using unbiased leave-one-dataset-out validation (Figure 1B)

1
2
3
4
5
6

Validate the final gene signature in independent validation datasets (Figure 2)

B) Kaplan-Meier analysis of datasets 1 to 6

   - Survival (%)
   - Time (Days)
   - \( n = 61 / 67 \)
   - \( P = 0.00968 \)

2. Crijns et al, 2009
   - Survival (%)
   - Time (Days)
   - \( n = 83 / 74 \)
   - \( P = 0.0135 \)

3. Yoshihara et al, 2010
   - Survival (%)
   - Time (Days)
   - \( n = 56 / 54 \)
   - \( P = 0.291 \)

   - Survival (%)
   - Time (Days)
   - \( n = 91 / 94 \)
   - \( P < 0.001 \)

5. Tothill et al, 2008
   - Survival (%)
   - Time (Days)
   - \( n = 71 / 69 \)
   - \( P = 0.00153 \)

6. TCGA 2011
   - Survival (%)
   - Time (Days)
   - \( n = 201 / 241 \)
   - \( P = 0.0144 \)
Survival Signature Performs Better Than Known Clinical Factors and Signatures

A) Meta-Analysis Signature

B) Signature + Stage + Debunking

C) Stage and Debunking only

D) TCGA Signature

E) Verhaak et al. Signature

F) Verhaak et al. Multivariate

G) Bentink et al, 2012

Crijns et al, 2009

Yoshihara et al, 2010

Mok et al, 2009

Bonome et al, 2008

Tothill et al, 2008

Dressman et al, 2007

Konstantinopoulos et al, 2010

Gillet et al, 2012

Yoshihara et al, 2012

Overall
Survival Signatures still not Clinically Relevant

Can we generate other clinically relevant signatures?
Debulking
Establishment of Debulking Signature

- Based upon the biologic basis of disease spread
- Analyzed 1525 microarrays of primary ovarian cancers
- 22% sub-optimal (>1CM)
- Supervised analysis/signature identification
- Generate pathway
qRT-PCR of 7 Pathway Genes Validates Signature And Provides an AUC of .8

A) qRT-PCR

B) qRT-PCR multivariate

AUC 0.8*

n = 78
Expression of Three Proteins Provides 93% Accuracy for Determining Sub-optimal Debulking Status
New Therapeutic Targets
Identification of “driver” events
through integrative genomics
Amplification in chromosome segment 5q31-qTER is significantly associated with poor survival

Birrer et. al, 2007, Journal of Clinical Oncology

Log-rank $P = .000252$

$n = 42$
Amplification of FGF18 and FGFR4 in chromosome 5

An overall 25% have DNA gain of Chr 5q31.3 to qTER

FGF18: 5q34
FGFR4: 5q35.2

70 patients from MGH (from 1991 to 2008)

Two individual cases:

Chromosome 5, Case 4595b
Chromosome 5, Case 4963a

* Log ratio of 1 indicates 4 copies
Uniqueness of FGF18 among the FGF18 family in the pathogenesis of ovarian cancer

**Table** Survival correlation of FGF family members in 53 microdissected late-stage high-grade papillary serous ovarian tumors

<table>
<thead>
<tr>
<th>Probe set (affy)</th>
<th>Gene name</th>
<th>Tumor / Normal</th>
<th>Cox regression</th>
<th>Probe set (affy)</th>
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**Conclusion:** FGF18 is the **ONLY** ligand out of the 18 members of human FGF family proteins showing both tumor related up-regulation and strong predicative power for poor prognosis.
FGF18 as a prognostic gene in high-grade papillary serous ovarian tumors

$p=0.001$

$p=0.026$

$p=0.031$

Mok et. al, n=53

Spentzos et. al, n=70

TCGA Project, n=200

Low FGF18 expression

High FGF18 expression
FGF18 promotes *in vivo* xenograft growth of SKOV3 cells

s.c. model (n=5)

![Graph showing tumor volume and weeks for SKOV3 pLoc-RFP and SKOV3 pLoc-FGF18 groups.]

i.p. model (n=5)

![Graph showing tumor mass and weeks for pLoc-RFP and pLoc-FGF18 groups.]

FGF18 levels in xenografts by IHC:

- Membrane positivity of FGF18
- Bar = 25 μM

![Images showing SKOV3-RFP and SKOV3 FGF18 membrane positivity.]

**SKOV3-RFP**

**SKOV3 FGF18**
FGF18 promotes tube formation of HUVEC cells

Baseline medium: EBM with 0.5% FBS
Assay for 2 hrs

Effect of FGF18 on HUVEC Tube Formation
FGF18 overexpression promotes \textit{in vivo} proliferation (Ki-67), angiogenesis (CD31) and tumor-associated macrophage infiltration (F4/80) in SKOV3 derived xenografts.

**IHC staining:**

<table>
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<tr>
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Bar = 50 μM, 100 μM, 100 μM respectively

T: tumor; S: tumor stroma
Potential mechanism of FGF18 mediated tumor progression in ovarian cancer cells

- Genes up-regulated genes by FGF18 are labeled in red
- Solid arrow = direct effects
- Dashed arrow = indirect effects
- Broken arrow = secretion
- Purple stick = binding

Diagram:
- Angiogenesis
- TAM infiltration
- Tumor endothelial
- IL8, IL6, CSF2, GRO1, GRO2, IL1A
- PI3K, GRB, SOS, Akt, Erk, NFκB
- Internalization
- Cytokine production
- FGF18, CXCR-2, VEGFRs, FGFRs, FRS2, GRB, SOS, Akt, Erk, NFκB, HMGA, PI3K, Cytokine production, TAM infiltration, Angiogenesis, Tumor endothelial, Tumor cell
Can the FGF-FGFR4 Axis be Targeted?

FGF Trap
Anti-FGF 18 Antibody
FGFR TKIs
Thank You