OVA1 — A New Blood Test for Ovarian Cancer Diagnostics

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Ovarian Cancer, Incidence and Mortality

Definition of ovarian cancer: Cancer that forms in tissues of the ovary. There are three types of ovarian cancer:

1. Epithelial (85-90%)
2. Germ cell tumors (5%)
3. Sex cord-stromal tumors (occur in connective tissue inside the ovary)

ACOG Education Pamphlet, 2010

Estimated new cases and deaths from ovarian cancer in the United States in 2009:

New cases: 21,550
Deaths: 14,600

National Cancer Institute, US National Institutes of Health

Ovarian Cancer

Incidence

Mortality

Data from the American Cancer Society
### Survival & Stage

**Invasive Epithelial Ovarian Cancer**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>5 Year Survival Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>The cancer is still contained within the ovary (or ovaries).</td>
<td>89%</td>
</tr>
<tr>
<td>II</td>
<td>The cancer is in one or both ovaries and has involved other organs (such as the uterus, fallopian tubes, bladder, the sigmoid colon, or the rectum) within the pelvis.</td>
<td>66%</td>
</tr>
<tr>
<td>III</td>
<td>The cancer involves one or both ovaries, and one or both of the following are present: (i) cancer has spread beyond the pelvis to the lining of the abdomen; (ii) cancer has spread to lymph nodes.</td>
<td>34%</td>
</tr>
<tr>
<td>IV</td>
<td>This is the most advanced stage of ovarian cancer. In this stage the cancer has spread to the surface of the liver, the lungs, or other organs located outside of the peritoneal cavity.</td>
<td>18%</td>
</tr>
</tbody>
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### Identifying the Question

- Early in the process, scientists at Vermillion, Inc. understood that the most important ingredient in a recipe is the unmet clinical need.

- Their initial instinct was to develop a test to screen for ovarian cancer in hopes of identifying the disease earlier.
  - After discussions with leaders working in this field, they came to understand that due to the low prevalence of ovarian cancer, development of a screening test was out of their reach.
  - These colleagues, however, suggested that their was a critical unmet need in the area of ovarian tumor triage.

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### Identifying the Question (Cont)

- Although ovarian tumors are relatively common, only a fraction of them are malignant.
  - 5-10% of women in the US will undergo a surgical procedure for a suspected ovarian neoplasm during their lifetime (ACOG)
  - 13-21% of these women will have an ovarian malignancy.
- They decided that being able to identify the malignant ones preoperatively would permit better preoperative management of women with ovarian tumors. Furthermore, women with a high likelihood of malignancy could benefit from referral to gynecologic oncologists.

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### So the Story Goes

- Although diagnostic test development remains challenging, novel technologies, including proteomics, genomics, and microRNA analysis, provide opportunities to identify biomarkers that in principle could accelerate the development of new diagnostic tests.

Identification of Biomarkers

Surface Enhanced Laser Desorption Ionisation Time Of Flight Mass Spectrometry (SELDI TOF MS)

The analysis of proteins by SELDI TOF MS can be divided up into 5 distinct phases:
1. Purification / enrichment of select proteins of interest on the array or chip surface.
2. The addition of a sample to existing spots on the sample on the chip surface and placement into the mass spectrometer.
3. The firing of a laser at the sample / matrix mixture.
4. Determination of the masses of the proteins in that sample.
5. Computer analysis and comparison of individual samples or groups of samples to look for changes in the expression of those proteins in a affected patient and control samples.

Purification / enrichment of selective proteins of interest on the array or chip surface.

Proteomics study design and results

<table>
<thead>
<tr>
<th>Total 593 samples from 4 different medical institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>370 Invasive ovarian cancer</td>
</tr>
</tbody>
</table>

Screening and cross validation

Purification and identification

Assay development

<table>
<thead>
<tr>
<th>Total 444 samples from fifth medical institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>47 ovarian cancer</td>
</tr>
<tr>
<td>20 breast cancer</td>
</tr>
</tbody>
</table>

Initial, Retrospective Studies Identify Preliminary Biomarker Candidates

Final 7 Biomarker Candidates Identified by SELDI-TOF:
1. ITIH4 – (inter-a-trypsin inhibitor heavy chain 4)
2. Transthyretin (prealbumin)
3. Apolipoprotein A1
4. Hepcidin
5. β2-microglobulin
6. Transferrin
7. CTAP3
Final Biomarker Candidates Plus CA125

Development of OVA1 algorithm
- Used two samples sets
  - Set 1: samples from University of Kentucky Medical Center (UK)
    - 284 subjects
    - 96 malignancies
  - Set 2: samples randomly selected from set collected under the OVA1 clinical study protocol
    - 125 subjects
    - 26% prevalence ovarian malignancy
- Pre-processing:
  - Log transformation for relevant markers
  - All normalized based on manufacturers’ suggested reference range

I think we have the right combination for the OVA1 algorithm.

Complementarity with CA125 and age
OVA1 Prospective Clinical Trial Performance

- Cutoffs chosen to emphasize sensitivity and NPV
  - False negative adversely impacts patient care since these would be missed cancers
  - False positive leads to surgery of benign case by gynecologic oncologist, which introduces no risk
- Output is index score from 0 to 10
  - >=5.0 in pre-menopausal subject indicates high likelihood of malignancy
  - >4.4 in post-menopausal subject indicates high likelihood of malignancy
- Caveats
  - Not a standalone test, i.e. to be used in conjunction with other findings (e.g. radiologic, clinical examination)
  - Not for screening
  - In conjunction with clinical assessment, both NPV and sensitivity >90%

Prevalence = probability of disease in the entire population at any point in time (i.e. 2% the U.S. population has diabetes mellitus)

Incidence = probability that a patient without disease develops the disease during an interval (the incidence of diabetes mellitus is 0.2% per year, referring only to new cases)

Sensitivity = probability of a positive test among patients with disease

Specificity = probability of a negative test among patients without disease

Negative predictive value = probability of no disease among patients with a negative test

In 2005, Quest Diagnostics Buys Share in Ciphergen (Now Vermillion, Inc.) for 15 Million Dollars

Acknowledgement

Eric T. Fung
Vermillion, Inc.
FMA Clear Vermoniion’s “OVA1®” Test To Determine Likelihood of Ovarian Cancer In Women With Pelvic Mass

Company Fined to Launch OVA1(TM)

FREMONT, Calif., Jan 22 (PRNewswire) -- Vermonion, Inc. (Pink Sheets: VNMN.OB)today announced it has successfully arranged for the first FDA-cleared test under Chapter 11 of the United States Bankruptcy Code at least 10 months following its filing for bankruptcy protection in the United States Bankruptcy Court for the District of Delaware. Vermonion emerged from bankruptcy with an unsecured interest in 18.9 percent of the company’s common stock and is now a fully operating company.

“Not everyone is more comfortable with our successful rebirth,” Vermonion’s legal advisor in connection with its successful reorganization efforts is Paul H. Rapoport, Rapoport, Rapoport & Rapoport LLP.

About Vermonion
Vermonion, Inc. is dedicated to the discovery, development and commercialization of novel high-value diagnostic tests that help provide physicians diagnosis and treatment outcomes for their patients. Vermonion, along with its outstanding scientific collaborations, has diagnostic programs in oncology, cardiology and women’s health. Vermonion is based in Fremont, California. Additional information about Vermonion can be found at the Web site of www.vermonion.com.

Human Epididymis Protein 4 (HE4)
Monitoring HE4 is a secreted protein that is over expressed and can be detected in high levels in women with ovarian cancer. It is cleared by the FDA for use to monitor recurrence or progressive disease in patients with epithelial ovarian cancer.

Monitor Ovarian Cancer using HE4

HE4 increases by 25% or greater in 66% of patients with progression of disease.

HE4 remains constant in 79% of patients without progression of disease.