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The Role of Biomarkers in the Management of Epithelial Ovarian Cancer

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Abstract

Ovarian cancer is the leading cause of death from gynecologic malignancies in the United States. Biomarkers have contributed to the management of ovarian cancer by monitoring response to treatment and recurrence, distinguishing benign and malignant pelvic masses and attempting to detect disease at an earlier stage. Levels of CA125 correlate with response to treatment and can rise 4.8 months prior to clinical disease recurrence. While initiating earlier treatment for recurrent disease may or may not improve survival, it can provide time to pursue multiple courses of novel or conventional therapy. As outcomes are improved when primary surgery is performed by a gynecologic oncologist, pre-operative discrimination of benign and malignant pelvic masses can facilitate appropriate referral. CA125, HE4, apolipoprotein A1, transthyretin, transferrin, and β 2-macroglobulin have contributed to the RMI, ROMA or OVA1 algorithms to distinguish benign from malignant disease. Difficulty detecting ovarian cancer at an early stage is a major factor contributing to poor clinical outcomes. Use of rising CA125 to trigger transvaginal ultrasound has provided adequate specificity in the NROSS and UKCTOCS trials and may produce as much as a 20% reduction in mortality in the latter trial with additional follow-up. New biomarkers and imaging are being developed.

Keywords

ovarian cancer; biomarkers; CA125; HE4; monitoring; pelvic mass; early detection; cancer imaging

INTRODUCTION

In the United States in 2016, ovarian cancer ranks fifth in cancer deaths among women and is the leading cause of mortality from gynecologic malignancies with 14,240 deaths and 22,280 new cases [1]. Worldwide statistics from 2102 estimate an order of magnitude greater impact with 238,700 new cancer cases and 151,900 deaths from the disease [2]. In the United States, the prevalence of ovarian cancer is 1 in 2500 for postmenopausal women

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above 50 years old and the lifetime risk of developing this disease is 1 in 70 for all women, compared to 1 in 8 for breast cancer [3]. Consequently, ovarian cancer is neither a common nor a rare disease.

Epithelial ovarian cancers exhibit substantial heterogeneity. Epithelial cancers that arise from the ovary develop from simple flattened epithelial cells that cover the ovarian surface or that line inclusion cysts. Surface epithelial cells transform into four distinct histotypes which resemble cells that line the normal fallopian tube (serous), endometrium (endometrioid), endocervical glands (mucinous), or that form rests within the vagina (clear cell) [4]. Ovarian cancer histotypes have been correlated with the abnormal re-expression of homeobox (Hox) genes such as *HOXA9*, *HOXA10*, and *HOXA11* that regulate normal gynecological organogenesis and differentiation [5]. A fraction of serous high grade cancers also arise from the fallopian tube [6].

Based on histological grade, genotype and molecular phenotype, ovarian cancers can be classified into two major groups. Type I low-grade carcinomas can be of serous, endometrioid, mucinous or clear-cell histotype. Low-grade ovarian cancers are most frequently diagnosed in early stage (I/II), grow slowly, and are resistant, but not refractory, to conventional chemotherapy [7]. Type I carcinomas generally have wild-type *TP53* and *BRCA1/2* with frequent activating mutations of *KRAS* (>50%) and inactivating mutations of *PTEN* [8]. The more prevalent type II high-grade carcinomas can include serous, endometrioid, or undifferentiated histotypes. High-grade cancers usually occur at an advanced stage (III/IV), grow aggressively, and respond to conventional chemotherapy [8]. In contrast to Type I carcinomas that are driven by genetic mutations, Type II cancers are generally driven by gene copy number abnormalities and by loss of tumor suppressor gene function such as *TP53* and *BRCA1/2*. High grade serous cancers consistently have *TP53* mutations (>96%). Germ-line or somatic mutations of *BRCA1/2* mutations are found in 20% of Type II cancers producing defects in homologous recombination repair (HRR) of DNA [8]. Other abnormalities in the HRR pathway account for “BRCAness” in >40% of epithelial ovarian cancers [3]. Activation of the PI3K pathway due to amplification or overexpression of genes in the PI3K family is associated with > 40% of type II cancers. Consequently, the discrepancy between type I and type II cancers offers the first step in understanding the heterogeneity of ovarian cancers and applying this new information to personalized cancer management [4,9].

Although five-year survival has improved over the last 3 decades, the overall cure rate of ovarian cancer remains approximately 30%. This disease has been named the “silent killer” based on the misconception that cancer becomes widespread without the occurrence of symptoms. In fact, more than 80% of cancer cases exhibit symptoms, even while the malignancy is still limited to the ovaries [10], but these symptoms are shared with a variety of more common benign gastrointestinal, genitourinary and gynecological conditions. When disease can be detected while still limited to the ovaries in stage I, up to 90% of patients can be cured with currently available surgery and chemotherapy. Even when disease has spread to the pelvis in stage II, 70% of patients can be cured, but disease that has spread throughout the abdominal cavity or beyond can be cured in less than 20% of cases. Currently, only 20% of cancer is detected in stage I and II by conventional examination and there is no

proven method for early detection of ovarian cancer. Computer modeling suggests, however, that detection of a greater fraction of cases in early stage could decrease mortality by 15–30% [11].

In this review, we briefly discuss the use of biomarkers to monitor cancer treatment, detect recurrence, refer patients with pelvic masses to appropriate surgeons, predict response to conventional or targeted therapy and develop methods for early detection.

MONITORING OVARIAN CANCER DURING TREATMENT AND FOR RECURRENCE

Management of ovarian cancer has improved over the last three decades, with an increase in 5-year survival from 38 to 46%, related to the more consistent use of cytoreductive surgery and combination chemotherapy with platinum compounds and taxanes [1]. Despite the improvement in overall survival, a fraction of patients with advanced stage disease fail to respond to primary therapy and at least 70% relapse. Biomarkers such as CA125 have been used to monitor response to treatment and to detect recurrence.

CA125

CA125 is a high molecular weight (5 million Dalton) heavily glycosylated transmembrane mucin (*MUC16*) which is overexpressed in 80% of epithelial ovarian cancers [12]. CA125 is cleaved just outside the cell membrane and is then shed into body fluids where it can be detected with double determinant immunoassays, providing the first clinically useful biomarker for monitoring the response to treatment of ovarian cancer [13]. While more sophisticated analyses have been developed [14], response to treatment is generally associated with a decrease in CA125 values by half, whereas a doubling of CA125 indicates drug resistance and disease progression. Persistent elevation of CA125 in patients after primary chemotherapy predicts persistence of residual disease with 90% accuracy [8]. CA125 can, however, return to normal during primary treatment and small amounts of persistent disease can be found in approximately half of second look operations. Consequently, CA125 is highly specific, but not optimally sensitive for monitoring a complete response to primary therapy.

Following initial cytoreductive surgery and combination chemotherapy, patients are generally observed for disease recurrence. Persistently rising CA125 is a highly specific indicator of recurrent disease. Rustin et.al. carried out a clinical trial where doubling of serum CA125 levels outside the normal range detected recurrent disease 4.8 months earlier than clinical signs and symptoms, but early treatment of recurrent ovarian cancer based on rising CA125 did not improve overall survival when compared to treatment at clinical relapse [15]. The strength of this conclusion is weakened by several limitations in trial design: (1) patients were not imaged consistently to identify persistent disease before randomization; (2) secondary surgical cytoreduction was not utilized at the detection of recurrence; (3) changes of CA125 within the normal range were not considered, delaying detection of recurrence; (4) treatment was delayed in a fraction of cases detected with CA125; and (5) taxanes were not used consistently in combination with platinum

compounds to treat recurrent disease, resulting in suboptimal therapy by current standards [16]. Consequently, only one quarter of patients detected by CA125 were treated promptly with a combination of drugs that could improve survival. In the United States, patients are generally monitored with CA125 at each return visit [17]. Doubling of baseline CA125 within the normal range prompts imaging to detect disease that might be resected during secondary cytoreduction. While it remains unresolved whether earlier detection of recurrence improves survival, patients have more time for advanced treatment with multiple conventional drugs and the opportunity to pursue novel agents while still asymptomatic and able to visit cancer centers that offer these agents. As more effective therapies are developed for recurrent ovarian cancer, monitoring will become more and more important.

HE4

Human epididymis protein 4 (HE4) is a 20–25 kDa protein that is secreted by epithelial cells and belongs to the family of whey acidic four-disulfide core (WFDC) proteins. Compared with its expression in normal tissues including ovary, the *WFDC2 (HE4)* gene is increased in the majority of ovarian cancers [18,19]. Hellstrom and colleagues were the first to demonstrate the potential of a serum HE4 protein level as a biomarker for ovarian cancer [20]. Moore, et. al., reported that HE4 had the highest sensitivity of 72.9% (at 95% specificity) among multiple markers including CA125 for distinguishing ovarian cancer from benign disease [21]. Combining CA125 with HE4 generated the highest sensitivity at 76.4% (at 95% specificity), suggesting that a combination of these two markers provides more accurate prediction for malignancy than either alone [21].

Several recent studies suggest that HE4 can serve as a biomarker for monitoring ovarian cancer treatment and recurrence. HE4 values decrease significantly from diagnosis of the disease (324.1 pM) to complete clinical remission (23.3 pM) [22]. HE4 may complement CA125 in monitoring a patient's response to treatment. One study utilized a four biomarker panel (CA125, HE4, MMP-7 and mesothelin) to monitor ovarian cancer patients after surgery and chemotherapy and examine the lead time of a rising biomarker levels before recurrence [23]. In 5 of 23 patients, HE4 levels were elevated before recurrence with a lead time of 4.5 months, similar to the lead time provided by CA125. HE4 levels increased prior to CA125 levels in an additional 2 of 5 patients. In 4 of 7 patients in whom both CA125 and imaging were negative, HE4 levels were elevated [23]. In a recent prospective trial, HE4 could identify recurrent ovarian cancers with 73.5% sensitivity and 100% specificity at a threshold of 70 pM. A combination of HE4 with CA125 improved overall sensitivity to 76.5 % with 100% specificity [24]. Consequently, HE4 can predict ovarian cancer recurrence earlier than CA125 in some patients and HE4 can be elevated in patients whose cancers fail to express CA125. A combination of CA125 and HE4 can provide slightly, but significantly better sensitivity for monitoring treatment and shorter lead time for detecting recurrent ovarian cancer than either marker alone.

DIFFERENTIAL DIAGNOSIS OF A PELVIC MASS

Each year in the United States a projected 289,000 women undergo exploratory surgery for a pelvic mass or an ovarian cyst [25,26]. For those women who have ovarian cancers,

specialized cytoreductive surgery is required to remove as much of the cancer as possible. In recent years, it has become apparent that the greatest benefit is achieved when there is no residual disease after cytoreductive surgery (R0) [27]. Benign lesions can be removed by surgeons with less extensive training. As management of ovarian cancer by gynecologic oncologists significantly improves patient outcomes, tests are needed that permit accurate referral of women with pelvic masses to the most appropriate surgeon. About 10% of pelvic masses are malignant in premenopausal women, compared with 20% in postmenopausal women [28]. In addition to menopausal status, imaging results and serum biomarker levels can help to distinguish benign from malignant pelvic masses. Currently, several algorithms have been established that combine age, menopausal status, imaging and serum biomarker(s) into a single index to estimate the risk of a mass being malignant.

Risk of malignancy index (RMI)

In 1990, Jacobs et.al., developed a risk of malignancy index (RMI) for evaluating the probability that a pelvic mass was malignant. The RMI is calculated by considering three pre-surgical features: ultrasound score (U, score = 0, 1 or 3); menopausal status (M, (premenopausal = 1 and postmenopausal = 3); and serum CA125 value (U/mL). The RMI is the product of $U \times M \times CA125$ [29]. In an initial study, RMI was calculated for 101 patients with benign disease and 42 patients with malignant pelvic masses. Using an RMI threshold of 200, the sensitivity was 85% and the specificity was 97% [29]. In multiple clinical studies, sensitivity for predicting ovarian malignancy has ranged from 71% (with 97% of specificity) to 88% (with 74% of specificity) [30,31]. Results of those studies suggest that RMI can distinguish ovarian cancer cases from benign lesions, but there is room for improvement.

Risk of malignancy algorithm (ROMA)

Moore et.al. evaluated several biomarker candidates for their potential to discriminate malignant from benign pelvic masses. A combination of CA125 and HE4 yielded the highest area under the receiver operating characteristic (ROC) curve ($AUC = 91.4\%$) [21]. Subsequently, CA125 and HE4 levels were integrated with menopausal status, but not imaging data, to develop the risk of malignancy algorithm (ROMA) [32]. In multiple clinical studies, the ROMA has provided 93% sensitivity at 75% specificity with a negative predictive value of 93% to 94%. Sensitivity of the ROMA for detecting malignancy has been lower in premenopausal women than in postmenopausal women. In a direct comparison, the ROMA proved superior to the RMI. At the same 75% specificity, the ROMA and the RMI reached 94% and 85% sensitivity respectively. In detecting early stage (I/II) cancers the ROMA detected 85% and the RMI detected 65% of cases [28].

In a second low-risk trial, the ROMA was calculated for 472 patients in the community, 89 of who actually had gynecologic cancers [33]. The algorithm exhibited 94% sensitivity at 75% specificity. The sensitivity was 100% in premenopausal patients and the negative predictive value reached 98%. Based on the success of this second clinical trial, the ROMA was approved by the Food and Drug Administration (FDA) in the United States in 2011 for distinguishing ovarian malignant from benign pelvic masses. Some subsequent studies have confirmed the predictive value of the ROMA [34–37], whereas others suggest that

combining CA125 and HE4 with the ROMA provides no more discrimination than either biomarker alone [38–40].

OVA1

OVA1 is another algorithm that was approved by FDA in 2009 to distinguish malignant from benign pelvic masses, permitting appropriate referral. The OVA1 is a multivariate index calculated by combining data from imaging, menopausal status, and CA125 with four other protein biomarkers including apolipoprotein A1, transthyretin, transferrin, and β 2-macroglobulin [41]. In this study, the OVA1 algorithm contributes 96% sensitivity at 28% specificity in postmenopausal women, compared with 85% sensitivity at 40% specificity in premenopausal women. The negative predictive value was 94% to 96%. An OVA1 registration study included 363 women with benign tumors and 161 with malignancies of which 151 were ovarian cancers. [42,43]. Overall, the OVA1 panel demonstrated higher sensitivity, but lower specificity than physician evaluation. Addition of the OVA1 panel enhanced the sensitivity for detection of malignant pelvic masses from 78% to 98%, but reduced specificity from 75% to 26%. A high negative predictive value of 98% was, however achieved with OVA1.

To date there has not been a direct comparison between OVA1 and the ROMA, but the algorithms appear to exhibit similar sensitivity. The OVA1 is markedly less specific than the ROMA (< 40% verse 75%). Both have 96% to 99% high negative predictive values. The difference in specificity should not influence patient outcomes as gynecologic oncologists can remove benign lesions, but use of OVA1 could impact distribution of medical resources, given the limited number of gynecologic oncologists. A second generation test for OVA1 is being developed with greater specificity.

Neither OVA1 nor ROMA should be used as a screening test for ovarian cancer. Neither should be used to delay planned surgery and should only be ordered to aid in the decision of whether or not to refer a patient to a gynecologic oncologist for specialized cancer surgery when there is a high probability that the pelvic mass is malignant. The real challenge is to refer a larger fraction of appropriate patients to gynecologic oncologists, as only approximately half of ovarian cancer patients have their initial surgery with a specialist trained to perform an optimal operation.

PREDICTION OF RESPONSE TO TREATMENT

A number of biomarkers have been reported to distinguish chemotherapy-sensitive from chemotherapy-resistant ovarian cancers (Table 1). None of these have sufficient predictive value to be clinically useful. Similarly, there is not a reliable biomarker or signature that predicts response or lack of response to paclitaxel. This is an important unmet need. In the GOG 132 clinical trial, patients with ovarian cancer that had been suboptimally cytoreduced were randomized to cisplatin, paclitaxel or a combination of the two agents [44]. Some 70% of patients responded to cisplatin alone or to a combination cisplatin with paclitaxel, but only 42% responded to paclitaxel alone. In subsequent large, randomized trials, a combination of platinum and taxanes produced longer overall survival than did platinum alone. Based on these data, essentially all patients are treated with both agents, despite the

fact that a majority of patients will experience toxicity from paclitaxel, but not benefit from the drug.

More than half of ovarian cancers from patients with germ-line BRCA1 or BRCA2 mutations that impair homologous recombination repair (HRR) of DNA will respond to PARP inhibitors. A fraction of cancers from patients with wild-type BRCA1/2 will also respond to PARP-inhibitors, presumably related to some other defect in HRR. Several groups are seeking a reliable test for BRCAness or PARPness that would predict response to PARP inhibitors, alone and in combination [3,45,46]. Measures of HRR deficiency have been evaluated as biomarkers for response to PARP inhibition [47]. Approaches have included: 1) sequencing of DNA repair genes seeking inactivating somatic and germ line mutations; 2) measurement of “genomic scars” produced by HRR deficiency including loss of heterozygosity (LOH); telomeric allelic imbalance (TAI) and large scale transitions (LST); 3) measurement of mRNA or protein expression for components responsible for HRR; and 4) functional assays of activation of the HRR machinery, double strand breaks or PARP activity [47]. Myriad Genetics has developed a test for HRR deficiency (HRD) that depends upon LOH, TAI and LST. In a recent randomized placebo controlled study, the PARP inhibitor niraparib prolonged progression free survival (PFS) in patients with platinum-sensitive ovarian cancers [48]. Patients with germ-line mutations of BRCA received the greatest benefit, but patients with BRCA wild-type cancers also had prolongation of PFS. Among patients with BRCA wild-type cancers, HRD-positive cancers were associated with greater PFS than HRD-negative cancers, but niraparib also improved PFS in the HRD-negative cases, suggesting that there is room for improvement in assays to predict response to PARP inhibitors.

Approximately half of patients with high grade serous ovarian cancers have abnormal activation of the PI3 kinase signaling pathway which should be necessary, but possibly not sufficient to predict response to inhibitors of PI3K, AKT or mTOR inhibitors. Efforts are also underway to define a signature for PI3Kness [49–51]. To date, however, there has not been a close correlation between assays of PI3K pathway activation and pre-clinical or clinical response to inhibitors of its component signaling molecules.

Approximately 8% of high grade cancers have overexpression of HER2 [52]. Anecdotally, ovarian cancers with HER2 overexpression have responded to trastuzumab, pertuzumab and trastuzumab emtansine. While HER2 testing is not done routinely in newly diagnosed ovarian cancer, it may be worthwhile in patients with recurrent ovarian cancer.

Relatively few high grade serous ovarian cancers express estrogen receptor (ER) or progesterone receptor (PR). A higher fraction of low grade ovarian cancers express ER and/or PR and can respond to hormonal therapy [53]. A recent report suggests that patients with low grade ovarian cancer who receive hormonal adjuvant therapy have better overall survival [54].

EARLY DETECTION

Significance

Although median survival for ovarian cancer patients has increased significantly over the last three decades, the overall cure rate has remained about 30% when all stages are included. One factor that contributes to this poor outcome is the late diagnosis of disease. At present, 75–80% of ovarian cancer patients are diagnosed with advanced stage (III/VI) where the cure rate is less than 20% [55–57]. If, however, the disease is diagnosed in Stage I or II, 70% to 90% of patients can be cured with conventional surgery and chemotherapy. Early detection could significantly improve clinical outcomes in ovarian cancer since computer models suggest that detection of early stage disease could improve cure rates by 15–30%.

Clinical requirements

The goal of early detection for ovarian cancer is to detect malignancy while still within the ovaries or limited to the pelvis, permitting earlier intervention to prolong overall survival and reduce mortality. Most advocates and clinicians believe that no more than 10 operations should be performed for each case of ovarian cancer detected (i.e., a positive predictive value (PPV) of >10%) [58]. Given the incidence of ovarian cancer, an effective screening strategy must have a high sensitivity of >75% for pre-clinical disease and an exceptionally high specificity of 99.6% to achieve a PPV of 10%. Current measurements and biomarkers for studies of early detection are discussed separately below. At present, no any single diagnostic modality (i.e., ultrasonography or serum biomarker CA125) has adequate sensitivity and specificity to meet these criteria.

Ultrasonography

In early studies, transabdominal ultrasonography (TAU) was used to detect ovarian cancer; however, the PPV was only 1.5%. With the development of transvaginal sonography (TVS), more precise imaging of the ovary was attained. Trials of TVS have been conducted in the United States, United Kingdom and Japan. Overall, the specificity of TVS is at the margin of that required to reach a PPV of 10% [59–63]. By contrast, studies of women screened with TVS and Doppler ultrasound at the University of Kentucky recorded a PPV of 14% [61]. In the larger PLCO and UKCTOCS screening trials, however, the PPV ranged from 1–2.8% for invasive cancers [64,65]. Remarkably, the sensitivity of TVS for detecting stage I ovarian cancer may not exceed 90%, particularly when screening prevalent disease. In the UKCTOCS trial, the sensitivity for detecting invasive disease of all stages in prevalent disease was 75% [66]. These studies suggest that ultrasonography may detect some cases of ovarian cancer at early stage, but lacks adequate specificity and sensitivity. Given the expense of TVS, at least in the United States, modeling of the potential cost and benefit exceeds the limits for other screening tests [67].

CA125

The evaluation of serum biomarkers for early detection of disease has largely focused on CA125 (MUC16) that can be monitored in serum. CA125 was initially detected by our group using the OC125 monoclonal antibody in a homologous double determinant assay

[13,68]. As the extracellular domain of CA125 contains multiple repeating subunits, OC125 could be used to trap the CA125 antigen and radiolabeled OC125 to detect trapped antigen. Subsequently, a heterologous double determinant assay was developed (CA125-II) using M11 antibody to trap antigen and OC125 antibody to detect the trapped antigen providing less day to day coefficient of variation ($< 5\%$) [69]. Overall, both CA125 assays reveal a sensitivity of 50–60% for stage I ovarian cancer and 90% for late stage disease. CA125 antigen levels can rise exponentially 12 to 24 months before diagnosis in a fraction of cases [70]. Although 80% of ovarian cancers express CA125, its determination on a single occasion lacks the sensitivity and specificity required for early detection. The specificity of CA125 is particularly problematic in premenopausal women where endometriosis, adenomyosis, and retrograde menstruation can produce false-positive elevations of antigen levels.

Combination or sequential use of CA125 and TVS improves specificity for early detection

The Prostate, Lung, Colon and Ovary Screening Trial (PLCO) had studied postmenopausal women between 55 and 74 years, randomizing 75,000 participants to a screening arm and a control arm in the trial [64,65]. For ovarian cancer screening, CA125 levels were measured upon entry into the trial and then measured annually for 5 years. TVS was performed upon entry into the trial and then repeated annually for 3 years. Participants had been followed for a total of 13 years. If CA125 became elevated or a pelvic lesion was encountered, participants were referred to their local physicians for management. A preliminary report showed that CA125 alone had a PPV of 3.7% for detecting ovarian cancer and TVS had a PPV of 1.0% which were clearly inadequate. The PPV rose to 23.5% when both tests were abnormal, but 60% of invasive cancers would not have been detected [64].

Specificity can be improved by screening with CA125 and TVS sequentially in a two-stage strategy. An early study in the United Kingdom by Jacobs, et al, randomized postmenopausal women to a control or screened group [71]. Among 10,985 women screened, 29 operations were performed to detect 6 cancers (PPV of 21%). During 7 years of follow-up, 10 more cancers were diagnosed in the screened group. During the same intervals, 21 ovarian cancers were diagnosed in the control group. Median survival (73 months) in the screened group was significantly greater than in the control group [72].

Use of rising CA125 to trigger TVS improves specificity

Serial monitoring of CA125 levels in blood can improve specificity. CA125 values rise exponentially in ovarian cancer patients reflecting exponential growth of the source of the antigen. In patients with benign disease, sequential CA125 values generally do not rise, even when CA125 is initially elevated (>35 U/mL). Algorithms have been developed that differentiate patients with ovarian cancer from those with benign disease or with no disease [73]. During a screening trial in Stockholm, rising CA125 achieved a sensitivity of 86%, a specificity of 99.7% and a PPV of 16% [74]. In subsequent studies, serial CA125-II values were analyzed with an improved risk of ovarian cancer algorithm (ROCA) that proved superior to a 30 U/ml cutoff for identifying women at increased risk [73,75]. The United Kingdom Trial of Ovarian Cancer Screening (UKCTOCS) accrued $>200,000$ postmenopausal women at average overall risk for ovarian cancer who were randomized to

three groups: (1) a control group of 101,359 followed by physical examination, (2) a group of 50,639 screened annually with TVS, and (3) a group of 50,640 screened annually with CA125 analyzed with the ROCA and followed by TVS in a fraction of cases. In the latter group, that considered each woman's previous values, TVS was prompted in about 409 from 50,078 participants (0.8%) [66]. Individuals with abnormal TVS were referred to surgery. Participants have been followed for 7 years. This trial was adequately powered to determine whether screening decreases mortality in patients with epithelial ovarian cancer. Data from 7 years of accrual suggest that this strategy could increase the fraction of patients detected with ovarian cancer in early stage (41.4%) with adequate sensitivity (85.8%) and specificity (99.8%) [70].

A much smaller study of 5,000 women conducted by the Normal Risk Ovarian Cancer Screening Study (NROSS) consortium coordinated by MDACC, attained similar specificity (99.9%) and positive predictive value (>30%) for serial CA125 values followed by TVS [76]. Used sequentially, a combination of CA125 analyzed with the ROCA and TVS clearly has adequate specificity for effective screening in a population at average risk for the disease. The critical question is whether this two stage strategy provides adequate sensitivity to produce a stage shift and improve mortality using CA125 alone for the initial stage.

Use of sequential CA125 analyzed with the ROCA followed by TVS improves sensitivity and may reduce mortality

In the NROSS study, more than 5000 postmenopausal women at average risk have been screened over 16 years using a two stage multi-modality strategy with annual CA125 followed by TVS in 1–2% of patients. Nineteen operations have been performed to detect 12 ovarian and 2 endometrial cancers. Of the 12 ovarian cancers, 10 have been invasive and 2 borderline. Nine of the 12 cases have been in stage I or II. In several cases, early stage cancers were detected by the ROCA when CA125 was still within normal limits. While these numbers are small, they do suggest that early stage cancer can be detected with this strategy.

Results of the UKCTOCS have been published within the last year and indicate that multi-modality screening using the ROCA may be associated with an improvement in mortality [77]. The ROCA detected twice as many cases as would have been detected with an arbitrary cut-off of CA125. Using pre-specified analyses that excluded prevalent cases and primary peritoneal disease, a 20% reduction in mortality was observed after seven years ($P<0.021$). There are, however, wide statistical bounds around this estimate which should narrow over the next 2–3 years. If the 20% reduction in mortality is maintained, the ROCA may provide a first-generation strategy that could be extended to the community. Results of the NROSS study suggest that this approach would be as feasible in the USA as in the UK.

Limitations of the Current Two-Stage Screening Strategy

Whatever the outcome of the UKCTOCS, there is clearly room for improvement in both biomarkers and imaging modalities. CA125 alone is not an optimal biomarker in that 20% of epithelial ovarian cancers express little, if any, CA125. Multiple biomarkers will almost certainly be required to detect all ovarian cancers. An additional challenge is posed by the

likelihood that at least a third of ovarian cancers may arise from the fimbriae of the fallopian tubes. Approximately 15% of ovarian cancers arise in the context of a BRCA1/2 germ-line mutation and as many as 80% of this fraction, i.e. 12%, are thought to arise from pre-malignant fallopian tube lesions that have been documented at prophylactic salpingo-oophorectomy. An additional 20% of high grade serous epithelial ovarian cancers only coat the ovary and have been described as primary-peritoneal cancers. Taken together, approximately a third of ovarian cancers may be of fallopian tube origin. If this is the case, there is no anatomic barrier to metastasis from the fimbriae. As soon as transformed cells can resist anoikis, metastasis could occur, quite possibly from very small lesions.

Development of “ovarian cancers” from the fallopian tube also poses a challenge for imaging, as TVS has limited resolution and has difficulty resolving the fallopian tube and particularly their fimbriae. Specificity is another limitation of TVS that can only partially be improved with Doppler ultrasound or micro-bubbles. The two stage strategy may overcome this limitation in part, because many benign lesions that do not produce continuously rising levels of CA125 and consequently may never be imaged. More sensitive and more specific methods of imaging are badly needed.

ENHANCING THE INITIAL STAGE OF EARLY DETECTION

New Protein Biomarkers

Over the last decade, several new ovarian cancer protein biomarkers have been discovered using multiple approaches, such as empirical development of monoclonal antibodies, gene expression arrays, lipomics and proteomics. For example, murine monoclonal antibodies have been prepared against mesothelin [78]. On ROC analysis, mesothelin has been shown to complement CA125 and to enhance detection of ovarian cancer when the two tests are used in combination [79]. Gene expression arrays have proven to be potentially powerful tools for biomarker discovery having contributed to the identification of HE4 [80–82], different kallikreins [83], prostasin [84], osteopontin [85], VEGF [86] and IL-8 [86]. Lysophosphatidic acid has been identified by lipomics as a new lipid biomarker [87]. Using proteomics, at least 11 candidates (IGFBP2, IGFBP3, KLK6, KLK7, KLK9, MDK, CA125, PROS1, SLPI, TIMP1 and HE4) have been discovered in ovarian cancer cell lines, and five candidates (GRN, IGFBP2, RARRES2, TIMP1 and CD14) have been identified in plasma from ovarian cancers including early stage patients [88,89]. Currently, the Early Detection Research Network (EDRN) website lists more than 200 ovarian cancer biomarker candidates. More large-scale clinical studies are required for evaluating those candidates as potential biomarkers.

Multiple biomarkers can increase sensitivity while maintaining specificity

Since only 80% of ovarian cancers and 50% of early stage patients express CA125, multiple biomarkers are needed to enhance detection of early stage and pre-clinical disease. During the last two decades, more than 50 biomarkers have been reported to improve sensitivity of CA125. However, the increased sensitivity achieved with biomarkers in combination has usually been associated with a marked decline in specificity. Improved mathematical techniques, such as neural network and mixed multivariate analysis, have permitted

improved sensitivity with panels of biomarkers, while maintaining specificity. When CA125, CA15-3, CA72-4 and M-CSF were assayed in sera from patients with stage I ovarian cancer and from healthy individuals, sensitivity could be improved from 48% with CA125 alone to 72% with artificial neural network analysis and to 75% with mixed multivariate analysis of the panel, while maintaining specificity at 98% [90,91].

In a collaborative study with the Ovarian SPOREs, 24 biomarkers including CA125 were assayed in 119 pre-diagnostic specimens from women who developed ovarian cancer while on the PLCO screening trial [92]. When evaluated with 95% specificity at the time of conventional diagnosis, CA125 could detect 73% of cases at all stages and 56% in early stage. In addition, CA125 detected 45% among the preclinical specimens. The best performing algorithm of 5 biomarkers (CA125, HE4, CA72.4, B7-H4 and CA15.3) improved the sensitivity for preclinical cases marginally from 41 to 47%, which did not achieve statistical significance [92]. This study suggests that multi-marker panels at a single point in time have not improved upon CA125 alone for detecting preclinical disease in early stage.

Autoantibodies

For early detection of cancer, the limitation of biomarker sensitivity is determined by the expression of antigen, the rate of antigen-shedding and the volume of cancer before it becomes capable of metastasis. Small volumes of cancer may not release adequate amounts of antigen to elevate serum levels, but could induce a human immune response [93]. Overexpressed, mutated or abnormally compartmentalized tumor-associated antigens in small volumes of cancer could stimulate a humoral immune response with measurable titers of specific antibodies against ovarian cancer associated antigens (autoantibodies) [94]. Consequently, autoantibodies against tumor-associated proteins might identify cancers that are too small to shed adequate amounts of protein biomarkers to be detected and improve lead time over conventional biomarkers such as CA125.

In recent years, several research groups utilized some new proteomic approaches to discover numerous autoantibody biomarker candidates for early detection of ovarian cancer (Table 2). Using proteomics, it is possible not only to identify new protein biomarkers, but also to find human immunoglobulin bound proteins and detect autoantibodies. Recent studies propose that anti-TP53 autoantibodies may serve as a biomarker for ovarian cancer [95,96]. The *TP53* tumor suppressor gene is mutated in virtually all high grade serous ovarian cancers [97]. Many *TP53* gene mutations cause stabilization and accumulation of TP53 protein in cancer cells and eventually break immune tolerance [98]. Previous studies showed that about 15% of ovarian cancer patients can be detected measuring autoantibodies against wild-type TP53 in sera, but most studies have only a restricted number of cases at the time of diagnosis [99]. Our recent studies with samples from the UKCTOCS subjects suggest that titers of anti-TP53 autoantibodies are present in as many as 25% of ovarian cancer patients and can rise 8–12 months prior to CA125 and more than 2 years before clinical presentation of CA125 negative cases [100].

MicroRNAs (miRNAs)

While most biomarkers for ovarian cancer have been proteins or autoantibodies, nucleic acids are also being evaluated. MiRNAs are small noncoding RNAs which downregulate protein expression of target genes by degrading their messenger RNA (mRNA) or interfering with translation of specific proteins. miRNAs have been implicated in the initiation, progression and metastasis of multiple cancers including ovarian cancer [101]. Several circulating miRNAs have been detected in the whole blood, plasma, serum and exosomes from ovarian cancer patients and have correlated positively or negatively with progression or chemo-resistance of ovarian cancers [102]. Several miRNAs have been proposed as biomarkers for early detection, diagnosis and prognostication of ovarian cancers, but none has been established to date [103,104].

Circulating tumor DNA (ctDNA)

ctDNA was first discovered in human blood in 1948 and in the blood of cancer patients in 1977 [105,106]. In recent years, clinical evaluation and application has been facilitated by development of techniques for rapid, deep sequencing of DNA in small quantities of plasma. Bettegowda *et al.* detected ctDNA pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers [107]. Pereira *et al.* demonstrated that the use of a personalized ctDNA profile as both a surveillance and prognostic biomarker in high-grade serous ovarian and endometrial cancers [108]. ctDNA had comparable sensitivity and specificity to CA125 and detected persistent cancer in 6 cases with negative CT scans [108]. ctDNA has also been detected in PAP smears [109] and in endometrial lavage fluid [110], although the number of early stage cancers detected to date is quite limited.

Exosomes

Exosomes are small (30–120 nm in diameter), but highly stable membrane vesicles that are released from a variety of normal and malignant cells [111]. Since the contents of exosomes (including cell-free DNA, mRNA, miRNA, proteins and metabolites) are derived from the cells from which they are released, it has been proposed that exosomes provide unique “signatures” of the cells from which they originate [111,112]. Kobayashi *et al.* correlated the invasive potential of ovarian cancer cells with levels of Let-7 miRNA and miR-200 in exosomes [113]. Using proteomic analysis, Liang *et al.* identified 380 previously unreported proteins that associated with cancer progression and metastasis [114]. Zhang *et al.* conducted nanoparticle tracking analysis to compare the characteristics of exosomes derived from normal ovarian epithelial cells and ovarian cancer cells. Exosomes derived from normal cells were larger than those derived from malignant cells [115].

Circulating tumor cells (CTCs)

CTCs were first reported in 1955 [116], but techniques for their efficient isolation and characterization have only been developed in the last decade by selecting malignant cells that express epithelial markers (e.g, EpCAM, cytokeratins, N-cadherin and/or vimentin) and excluding cells that express the hematopoietic cell marker CD45 [117]. Substantial research has focused on the application of CTCs for diagnosis, prognostication and measuring

response to chemotherapy in breast cancer, prostate cancer, colorectal cancer and lung cancer [118]. For ovarian cancer, changes in the levels of CTCs have been proposed as prognostic biomarkers to predict platinum resistance and prognosis [119,120]. In addition, fluorescent silica nanoparticles have been used for one-step detection of ovarian cancer CTCs [121]. The limited numbers of CTCs that can be isolated from small amounts of blood argue against their use as a biomarker for early detection.

ENHANCING THE SECOND STAGE OF EARLY DETECTION

New Imaging Technologies

Although TVS can improve early detection of ovarian cancer using a two-stage (CA125/ROCA with TVS) screening strategy [76], it is still not an optimal second stage due to limited sensitivity and specificity and to an inability to visualize small lesions of the fallopian tube. Therefore, it is necessary to improve imaging techniques.

Measurement of aberrant angiogenesis

All cancers must develop neovasculature in order to grow and progress. Vessels and blood flow differ between benign and malignant pelvic masses and these differences have been measured at the level of macro-vessels (100–200 μ M) by 3D color-Doppler sonography. Over the last decade, perfluorocarbon containing microbubbles that are one half to one third of the size of erythrocytes have been used to enhance and to measure blood flow in benign and malignant pelvic lesions at the level of capillaries [122]. Contrast enhancement with pulse-inversion harmonic TVS imaging has further improved the specificity of distinguishing malignant from benign pelvic masses by measuring greater peak enhancement and more delayed wash-out in the malignant lesions. Investigators have conjugated microbubbles with knottin peptides that bind to $\alpha_v\beta_3$ integrins on endothelial cells [123]. Knottin-conjugated microbubbles produced a greater signal in human ovarian cancer xenografts than did the non-coated microbubbles, suggesting that sensitivity of contrast enhanced TVS might be enhanced. Translation to clinical screening must still be achieved.

Superconducting Quantum Interference Device (SQUID) sensor

SQUID technology provides an extraordinarily sensitive method to detect changes in magnetic fields. When ferritin nanospheres are free-floating in the blood or peritoneum, there is no delay in magnetic relaxation after a magnetic pulse is passed through the ovary. If, however, antibody-coated ferritin nanospheres are attached to ovarian cancer associated antigens on the surface of tumor cells, a significant delay in magnetic relaxation is observed. Preliminary studies have detected 10^6 SKOv3 ovarian cancer cells in culture or in xenografts using ferritin nanospheres conjugated with antibodies against CA125 [124,125]. Using an ovarian phantom placed at typical ovarian depths, high spatial resolution has been reached with 5×10^6 ovarian cancer cells. Compared with current imaging such as CT, MRI and PET-CT scan which provide the best detection of 3–5 mm cancer nodules, SQUID imaging promises to improve sensitivity by one to two orders of magnitude. Efforts are underway to replicate and extend these observations.

Auto-fluorescence imaging

A new optical imaging system can identify pre-invasive and occult cancers in human fallopian tube epithelium utilizing light-excited endogenous fluorescence (auto-fluorescence). A recent study reported that sensitivity and specificity from optical images of 56 cases were 73% and 83% respectively for the entire cohort. In addition, positive and negative predictive value (PPV and NPV) were 57% and 91% [126]. The result suggests that this technology might be used to identify malignant fallopian tube lesions at an early stage. Falloposcopy could be used periodically to examine tubes, which would be particularly relevant for women with BRCA1 or BRCA2 germ line mutations who wanted to complete their families before elective prophylactic salpingo-oophorectomy.

Superparamagnetic iron oxide nanoparticles (SPIONs) with Magnetic Resonance Imaging (MRI)

SPIONs are commercially available agents for improving image contrast with MRI. SPIONs have been conjugated with the C595 monoclonal antibody that binds to the MUC1 antigen on ovarian cancer cells. SPIONs-C595 probes exhibited good tumor accumulation, no cytotoxicity, and potential positive selectivity for MUC1-overexpressing ovarian cancer cells [127,128]. However, a recent *in vivo* study from another group showed very low accumulation of ultra-small super paramagnetic iron oxide nanoparticles-C595 probes in the targeted site [129]. The charge for MRI imaging may prohibit cost-effective screening.

PERSPECTIVE

Improvement in the outlook for patients with ovarian cancer has developed slowly. Over the last three decades, five-year survival has improved substantially from 37% to 46% related, in all probability, to use cytoreductive surgery and combination chemotherapy [1]. The role of biomarkers in improving survival is more difficult to document.

Since it was introduced in 1983, CA125 has contributed worldwide to the care of hundreds of thousands of women with ovarian cancer. CA125, HE4 and other biomarkers have been utilized in the RMI, ROMA and OVA1 algorithms to identify patients who would benefit from the surgical skills of a gynecologic oncologist. Recent appreciation that complete resection of all visible ovarian cancer is associated with the best prognosis, makes appropriate referral all the more important. Rising CA125 has signaled resistance to chemotherapy and has undoubtedly prevented needless toxicity of ineffective chemotherapy. CA125 can detect disease recurrence at least 4.8 months before signs and symptoms develop clinically. If progressive increases of CA125 within the normal range of values (<35 U/mL) are taken seriously, much greater lead time may be possible. The challenge is not the ability of the biomarker to detect disease recurrence, but rather the effectiveness of treatment for recurrent disease. As targeted therapy for recurrent ovarian cancer improves, detection of recurrent disease at an earlier interval will become more important. With acceleration in the acquisition of new knowledge and the discovery of more effective cancer treatment, it would seem prudent to monitor patients for disease recurrence to permit as much time as possible to access new treatments. New biomarkers including HE4, autoantibodies and ctDNA may contribute not only to earlier detection of primary ovarian cancer, but also to earlier

detection of recurrent disease. Predictive biomarkers will aid in choosing more effective therapies for recurrent disease, but will also affect the choice of primary therapy.

Perhaps the most important application of biomarkers will be in early detection of ovarian cancer that could decrease mortality by 15–30%. While targeted therapy promises to prolong survival in the short run, it remains to be seen how rapidly an impact on mortality will be achieved. For early detection, two stage strategies appear most promising. The initial stage must be improved. Our recent studies suggest that a combination of CA125, HE4 and CA72.4 will detect 18% of cases missed by CA125 alone [130]. More dramatic improvement may be seen with a panel of autoantibodies including anti-TP53. As important, will be improvements in the second stage of detection, improving on TVS. Other new imaging techniques, such as contrast-enhanced transvaginal sonography, SQUID, and auto-fluorescence may provide the much greater sensitivity needed to image or detect small volume disease in the fallopian tube before it metastasizes. Each of these areas can and will be developed.

EXPERT COMMENTARY

Over the last three decades, there has been progress in the application of biomarkers to the care of patients with ovarian cancer, but there are still many unmet medical needs to which biomarkers could contribute. CA125 has permitted monitoring of response to primary chemotherapy and to conventional and novel agents used to treat recurrent disease. With regard to monitoring response to initial chemotherapy with carboplatin and paclitaxel, CA125 has proven to be a highly specific biomarker, but lacks sensitivity to detect persistent disease within the peritoneal cavity found at “second look” operations in 50% of cases with a “normal” CA125. Use of multiple biomarkers, including HE4, might enhance the sensitivity of CA125 in this setting. Obtaining relevant clinical information regarding the continued presence of cancer has hindered evaluation of multiple biomarkers. In the absence of promising therapies for “positive” second-looks, this procedure had been abandoned, but with the development of immunotherapy that could be most effective against small volumes of residual disease and with the discovery that residual deposits of drug-resistant ovarian cancer are undergoing autophagy in more than 80% of cases, second-look operations could be reinstituted in research centers conducting trials of immunotherapy and anti-autophagic therapy. Protein biomarkers, auto-antibodies and ctDNA can be assayed from blood obtained prior to positive and negative second look operations, permitting the evaluation of novel combinations of biomarkers. Validation of these panels could eliminate the need for second look procedures going forward, while identifying patients certain to progress who would be candidates for novel therapies.

Monitoring of ovarian cancer patients for disease recurrence with CA125 has been challenged based on a single trial that had significant limitations as discussed in this review. CA125 had accurately tracked disease recurrence and provided 4.8 months of lead time. Moving forward, greater lead time could be obtained by monitoring the trend of CA125 within a normal range. Autoantibodies, shed proteins and ctDNA could be evaluated to further improve the accuracy and lead time of CA125, but the greatest need is to develop more effective therapy for recurrent disease. One of the best examples of the interaction of

molecular diagnostics and therapeutics is the use of hCG to monitor recurrence of gestational trophoblastic disease that can be cured even after recurrence with available therapy. If we are to improve outcomes for women with ovarian cancer, participation in clinical trials is essential and these trials may well be the most promising treatment for women with recurrent disease. At present only 3% of patients participate in clinical trials within the United States. With targeted therapy, anti-angiogenic therapy and immunotherapy there are many new and promising options for women with recurrent ovarian cancer. By waiting for symptomatic recurrence of bulky disease, we minimize the chance that patients would be able to participate in these studies, or, in many cases, even receive an adequate trial of the several conventional therapies that, if active, could prolong useful survival.

Three algorithms have been developed that distinguish benign from malignant pelvic masses to permit appropriate referral to gynecologic oncologists with the specialized training to perform cytoreductive operations. Two have been FDA approved. It is clear that patients who can have all visible tumor removed have the best prognosis and outcomes for women who receive their care from gynecologic oncologists are improved. At present, less than half of patients with ovarian cancer are referred to gynecologic oncologists for their care. Despite the availability of effective biomarkers, other factors prevent effective referral.

For personalized or precision therapy, biomarkers that predict response to particular agents will be required. To date, we do not have clinically useful tests that predict response to carboplatin, paclitaxel, liposomal doxorubicin or gemcitabine. In the case of carboplatin and paclitaxel, development of biomarkers for response to the individual agents has been complicated by the fact that previously untreated patients generally receive both carboplatin and paclitaxel simultaneously. This is particularly problematic in the case of paclitaxel where less than half of patients are likely to benefit from the drug based on the GOG132 trial. For targeted therapy, companion diagnostics are being developed for PARP inhibitors. Clinically useful predictive tests are not yet available for MEK inhibitors in low-grade cancers with K-Ras mutations or for response to mTOR, AKT or PI3K inhibitors in high-grade cancers with activation of PI3K signaling. Given the potential toxicity and expense of check-point inhibitors, biomarkers with negative predictive value would be valuable.

At present there is no established screening strategy for early stage ovarian cancer in women at average risk for developing ovarian cancer. The two-stage UKCTOCS trial shows promise in that as much as a 20% reduction in mortality was observed. Both the UKCTOCS trial and the NROSS trial suggest that no more than 3–4 operations would be required for each case of ovarian cancer detected, so this strategy should be feasible in developed countries, if the mature data continue to show this magnitude of difference. Clearly there is room for improvement in both stages of the strategy as outline in the review.

Consensus panels have recommended quarterly or semi-annual screening with CA125 and TVS in women with germ-line BRCA1 or BRCA2 mutations, prior to prophylactic risk-reducing salpingo-oophorectomy. Data that screening improves survival have been difficult to obtain. Virtually all of the ovarian cancers in this group are Type II high-grade serous malignancies that exhibit *TP53* mutations. As many as 80% of the cancers in this group may arise from the fimbriae of the fallopian tube. DNA with mutant *TP53* has been obtained

from cervical secretions in cases of advanced-stage ovarian cancer. Serial monitoring of patients for mutant TP53 in ctDNA in blood and the cervix might aid in the detection of early lesions.

FIVE-YEAR VIEW

Over the next five years, the development of biomarkers for ovarian cancer will evolve with the development of more effective targeted therapies and immunotherapies for the disease. For low-grade Type I ovarian cancers, given the prevalence of activating K-Ras mutations (>50%), MEK inhibitors will be evaluated in the clinic. Biomarkers will be sought in tissues from these patients before, during and after therapy that will identify mechanisms of resistance to MEK inhibition which could be overcome with effective combinations of targeted agents. For high-grade Type II ovarian cancers, genetic signatures and panels of biomarkers will be developed that more accurately predict response to treatment with PARP inhibitors in order to identify the 40–50% of ovarian cancers that would respond. Combinations of PARP inhibitors with other targeted drugs will be evaluated and biomarkers chosen that will guide the selection of these combinations. The Combinatorial Adaptive Resistance Therapy (CART) platform developed by Gordon Mills and colleagues should prove helpful. In this approach, a single targeted drug such as a PARP inhibitor is used to treat cancer cells in 3-D culture or in patient derived xenografts. Cells are analyzed before and after treatment with reverse phase protein arrays to identify signaling proteins in resistance pathways that are adaptively activated in response to treatment with the agent. Inhibitors of the upregulated pathways are then paired with the PARP inhibitor. A similar approach can be applied to biopsy obtained before and during treatment in patients on clinical trials. This approach has already been utilized to choose agents to combine with PAR-inhibitors in Phase I-II clinical trials. If immunotherapy with check point inhibitors directed against CTLA4, PD1 and PDI ligand has a significant impact on a fraction of patients with ovarian cancer, biomarkers will be needed to identify those individuals, given the expense and toxicity of these agents. Biomarkers with high negative predictive value could be particularly helpful.

As the UKCTOCS data mature, if the 20% reduction in mortality is maintained, screening with annual CA125 and the ROCA could be implemented in developed nations. Progress should also be made in improving both the first and second stages of two-stage strategies for early detection. A New Risk of Ovarian Cancer Algorithm is being developed with CA125, HE4, CA72.4 and anti-TP53 autoantibodies and will be tested for specificity in a multi-center clinical trial adequately powered to predict positive predictive value and specificity with narrow bounds. Anti-TP53 can detect ovarian cancer 8–12 months prior to detection with CA125 and two years prior to conventional diagnosis in patients whose cancers do not express CA125, but only 25% of ovarian cancers are associated with anti-TP53 autoantibodies. Over the next 5 years substantial progress should be made in identifying a panel of 4–5 other autoantibodies that could detect ovarian cancers missed by anti-TP53 autoantibodies or CA125. Progress should also be made in improving the sensitivity of imaging in the second stage of a two-stage strategy. Modalities such as magnetic relaxometry made possible by SQUID sensing could be transformative.

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Key issues

- Despite improvement in 5-year survival over the last 3 decades through cytoreductive surgery and combination chemotherapy, ovarian cancer remains the most lethal gynecologic malignancy, claiming 14,240 lives annually in the United States. When all stages are considered, ovarian cancer can be cured in only 30% of cases.
- CA125 has provided a useful biomarker for monitoring response to treatment in more than 80% of ovarian cancer patients. CA125 is a highly specific biomarker for persistent or growing disease, but is not optimally sensitive for early stage disease or small volumes of cancer that remain on the peritoneum after primary treatment.
- Rising CA125 can detect disease recurrence more than 4.8 months prior to clinical diagnosis. While it remains unresolved whether earlier detection of recurrence improves survival, patients have more time for advanced treatment with multiple conventional drugs and the opportunity to pursue novel agents while still asymptomatic and able to visit cancer centers that offer these agents. As more effective therapies are developed for recurrent ovarian cancer, monitoring recurrence will become more and more important.
- Biomarker algorithms have been developed with CA125 (RMI), CA125 plus HE4 (ROMA) and CA125 plus four other biomarkers (OVA1) to identify patients who should be referred to specially trained gynecologic oncologists for their primary surgery which has been shown to improve outcomes.
- While biomarkers are not yet available to guide the use of conventional chemotherapy with carboplatin and paclitaxel, progress has been made in developing predictive tests for response to PARP inhibitors.
- At present, screening is not recommended for ovarian cancer in women at average risk for the disease, but development of an effective strategy could significantly impact mortality.
- Ovarian cancer is neither a common nor a rare disease with a prevalence of 1 in 2500 for postmenopausal women in the United States and a lifetime risk of 1 in 70. Consequently, an effective screening strategy that must not only be sensitive (>75% for pre-clinical disease), but also highly specific (99.6%) to achieve a positive predictive values of 10%, i.e., 10 operations for each case of ovarian cancer diagnosed.
- Two stage screening strategies are most promising where year-to-year changes in CA125 prompt transvaginal ultrasound. Trials in the United States (NROSS) and in the United Kingdom (UKCTOCS) have demonstrated that this strategy is adequately specific with 3–4 operations for each case of ovarian cancer diagnosed.

- Results from the UKCTOCS trial suggests that two-stage screening could reduce mortality by 20%, but another two years will be required for data to mature.
- Opportunities exist to improve both the first and second stages of screening.

Table 1

Examples of proposed biomarker candidates for distinguishing chemotherapy-sensitive from chemotherapy-resistant ovarian cancers

Study, year	Number of case	Biomarker candidate	Samples	Resistance to chemotherapy	SEN	SPE	PPV	NPV	Ref
Kamazawa et al., 2002	27	MDR-1 gene	Frozen tissue	paclitaxel-based	95%	100%	96%	NS	[131]
Hartmann et al., 2005	79	The 14-gene predictive model	Frozen tissue	Platinum & Paclitaxel	86%	86%	95%	67%	[132]
Helleman et al., 2005	96	Multiple predictive genes algorithm	Frozen tissue	Platinum-based	89%	59%	24%	97%	[133]
Gevaert et al., 2008	49	Microarray analysis	Frozen tissue	Platinum-based	67%	40%	NS	NS	[134]
Williams et al., 2009	143	Multivariate gene expression models (GEM)	NS	Carboplatin	77%	56%	71%	78%	[135]
Ferriss et al., 2012	55	COXEN gene expression profiling predictors	FFPE	Carboplatin	91%	17%	60%	57%	[136]
Angioli et al., 2014	76	HE4 protein	Serum	Platinum-based	83%	87%	86%	85%	[137]

NS: not specified; SEN: sensitivity; SPE: specificity; PPV: positive predictive value; NPV: negative predictive value; FFPE: formalin-fixed and paraffin-embedded

Table 2

Recent discoveries of autoantibody biomarker candidates for ovarian cancer by systemic approaches

Study, publication year	Number of case/benign/control	Technology	Autoantibody candidate	Ref
Anderson et al., 2015	34/0/30	high-density programmable protein microarray (NAPPA)	ACSBG1, AFP, CSNK1A1L, DHFR, MBNL1, TP53, PRL, PSMC1, PTGFR, PTPRA, RAB7L1 and SCYL3	[138]
Karabudak et al., 2013	20/20/20	iTRAQ and protein microarray	CFL1, EZR, HIST1H1C, HNRNPAB, HSPA9, PDZD11, PFN1, PP1A, SERF2, TUBA1C and JUP	[139]
Murphy et al., 2012	38/0/15	High content human protein array	TP53, ADD1 and ENSA	[140]
Tang et al., 2010	11/0/11	Reverse capture antibody array	35 candidates (see reference)	[141]
Gnjatic et al., 2010	51/0/53	ProtoArray human protein microarray	UBTD2, TGIF2LX, TSC22D4, MYST2, CCDC44, TGIF2, DRAP1, NPM3, FGFR1, TP53, UBL4A, FER and ZNF434	[142]
Gunawardana et al., 2009	30/0/30	ProtoArray human protein microarray	15 candidates (see reference)	[143]