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Biomarkers of ovarian tumours

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Abstract

Ovarian cancer is one of the most aggressive gynaecological malignancies and most often the high mortality is a direct result of delays in diagnosis. The development of an ovarian cancer-specific biomarker for the early detection of disease has the capacity to improve the dismal survival rate. Currently, there are multiple investigations that are utilising both genomic and proteomic technologies to identify genes, gene products and proteins that may potentially identify diagnostic ovarian cancer biomarkers. Here, we review the studies that are involved in biomarker development for the detection of ovarian cancer.

Keywords: Biomarker; Cancer; Microarray; Ovarian; Proteomics

1. Introduction

It is estimated that 25 400 women will be diagnosed with ovarian cancer in 2003 and 14 300 will succumb to the disease [1]. The mortality associated with this malignancy begets a huge burden such that it accounts for the greatest number of fatalities among all gynaecological cancers [2]. Unfortunately, the majority of epithelial ovarian cancers remain clinically undetected until patients have developed late stage disease and only a mere 25% of cancers are detected as stage I disease [2]. Once stage III and IV ovarian cancer, which is defined by peritoneal and extra peritoneal metastatic spread, is diagnosed, the survival decreases from 95% at stage I to approximately 20–25% five-year survival despite appropriate treatment [3–6]. Therefore, clinical outcome and possibly survival may be significantly improved by the identification of stage I disease without the need to change surgical or chemotherapeutic approaches. One of the obstacles that is encountered when attempting to identify disease that is confined to the ovary is that affected patients rarely become symptomatic in the early stages. In contrast to the breast, prostate and colon, the ovary is anatomically more difficult to assess during a routine physical examination and bimanual pelvic examination has not proved to be an effective method of identifying incident cases of early stage disease [7]. Furthermore, non-invasive radiographic assessments alone, such as ultrasonography, frequently cannot distinguish between a benign condition and a malignant lesion, which often results in subsequent costly and unnecessary surgical interventions [8–14]. These difficulties along with the relative rarity of ovarian cancer such that the prevalence in the general population is only 40 cases per 100 000 women older than 50 years, complicates the ability of clinicians to screen for and detect preclinical disease in both premalignant and early stage epithelial ovarian carcinoma.

1.1. Biomarker development

With regards to the early detection and prognostication of ovarian cancer, the need for and development of reliable serum biomarkers, which are both sensitive and specific, remains a long awaited priority. Investigators are aware of this need and the Early Detection Research Network (EDRN) established by the National Cancer Institute have proposed 'guidelines' for the development

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of screening biomarkers [15]. The EDRN delineates a schema with five phases of screening biomarker development, which hopefully will lead to the coordination of various institutions and culminate in the application of a systematic, efficient and effective biomarker-based screening programme. These phases include preclinical exploration, clinical assay and validation, retrospective and prospective screening, as well as conducting a clinical randomised trial for the purpose of assessing the endpoints of cancer screening [15]. When evaluating a biomarker for possible presymptommatic screening, one must consider the low prevalence of ovarian cancer in the general population. Only 1 in 2500 postmenopausal women are affected annually [2]. This number seems rather low in comparison to the 212 600 women that will be diagnosed with breast cancer in that same period of time [National Cancer Institute (NCI). Cancer Net PDQ cancer information summaries, monographs on "screening for breast cancer" (available at http://www.cancer.gov/cancerinformation/pdq/)]. The specificity of a putative biomarker is challenged by this low incidence. Ovarian cancer is most often definitively diagnosed with a laparotomy, and because this process is costly on many levels, a biomarker with a low specificity cannot be tolerated. A requirement for a specific biomarker has the following statistical profile: a positive predictive value of 100%, a specificity of 99.6% and a sensitivity of 100% [5]. Based on these characteristics, a positive biomarker result will subject ten women to undergo diagnostic exploratory laparotomy or laparoscopy with only one case of ovarian cancer being established.

2. Beyond CA-125

For a widespread screening programme to be successful in reducing the mortality associated with ovarian cancer, recognition of early-stage disease must be targeted. Unfortunately, CA-125, a high molecular weight glycoprotein and serum biomarker [16], does not fit the aforementioned statistical profile, but is approved for monitoring recurrence of disease [4,5,17]. Clinically significant elevations in CA-125 occur in only 80–85% of women diagnosed with early stage disease and 50–60% of late stage disease. Furthermore, CA-125 can also be increased in a variety of other conditions, both benign and malignant, such as the first trimester of pregnancy, breast cancer and endometriosis, as well as lesions that promote any type of peritoneal irritation [18–20]. Recently, several studies have investigated the potential use of CA-125 in combination with other serum markers and/or transvaginal ultrasonography [10,11,21]. Most of these combinations consisted of markers that are high-molecular weight glycoproteins similar to CA-125, such as CA 15-3, CA 72-4, CA 19-9, Lipid-associated sialic acid (LASA) and OVX1 [22–26]. Some of these biomarker combination strategies have resulted in increased sensitivities at the cost of specificity and, as discussed earlier, this profile is not predictive of disease in screening for early stage ovarian cancer [23,26]. However, one such study focused on evaluating the combination of CA-125, OVX1, LASA and CA72-4 in the setting of a pelvic mass and found that by using a logistic regression analysis, a specificity of 93% could be achieved compared with 76.8% with CA-125 alone [25]. With regards to ultrasonography and CA-125, a study involving more than 22 000 postmenopausal women either over age 45 years or more than 1 year from their last menstrual period, were followed-up for elevated screening levels of CA-125 with transvaginal ultrasonography. Despite significant increases in the median survival between the screened groups from the controls, there was not a significant difference in mortality [5]. There is also evidence that screening for ovarian cancer with serial levels of CA-125 measured over time and interpreted with the risk of ovarian cancer (ROC) calculation may be an optional method to detect preclinical disease [27].

Even though many investigational serum biomarkers such as prostasin and osteopontin [28,29] are emerging as promising answers to this query, new and exciting approaches to cancer diagnosis and screening are on the horizon, such as transcriptional profiling and proteomics [30,31]. Proteomics involves the analysis and illumination of all proteins encoded by the genome. It is these proteins that can be pursued as biomarkers for screening and cancer detection and possibly elucidate the molecular mechanisms involved in oncogenesis.

3. Molecular investigation

3.1. The proteomic horizon

At the NCI–Food and Drug Administration (FDA) Clinical Proteomics Program, a proteomic approach is being investigated for use in the early detection of epithelial ovarian cancer, as well as a variety of other processes such as auto-immune diseases, infectious diseases, prenatal diagnosis, transplantation rejection and vascular disease [32,33]. The proteomic approach has also been investigated for the diagnosis of breast [34], prostate [35] and liver cancers [36]. The complexity and enormity of analysing the proteome is recapitulated in the size and heterogeneity of the proteome itself. Proteins may exist in a variety of modified forms with a conservative estimate of the number of different protein species being greater than 300 000 [37]. Therefore, the identification of appropriate molecular markers, that are both sensitive and specific for epithelial ovarian cancer, is of paramount importance for the NCI–FDA Clinical

Proteomics Program. A clinical trial has been proposed which hopefully will lead to the development of the first reliable screening test for ovarian cancer for the general population. Petricoin and colleagues conducted protein-profiling experiments and generated proteomic spectra by mass spectrometry (surface-enhanced laser desorption and ionisation (SELDI)) [38] and an analytical tool known as a genetic algorithm or artificial intelligence computer algorithm, which discriminates protein patterns into 'diseased', and 'unaffected' training sets (Fig. 1) [38,39]. These experiments are based on the premise that pathological changes in tissue such as the ovary will be reflected in a particular and potentially diagnostic protein 'biosignature' pattern in serum [38–40]. Using this method, low molecular weight serum protein profiling was performed and a specific protein signature was identified that was associated with ovarian cancer from asymptomatic women with a high risk of developing ovarian cancer, such as patients with *BRCA1* or *BRCA2* mutations, which were provided from the National Ovarian Cancer Early Detection Program (NOCEDP) clinic at Northwestern University Hospital (Chicago, IL, USA) [38,39]. The study comprised analysing and distinguishing a serum protein signature specific for ovarian cancer based on a 'training set' of serum samples from 50 healthy and 50 women with ovarian cancer (Fig. 2). The bioinformatic program was 'trained' with known mass spectrometry data from women who were unaffected with ovarian cancer and from a cohort of women who were affected with ovarian cancer at various stages of the disease process, from whom blood was obtained prior to surgical or chemotherapeutic intervention. An additional 116 unknown serum samples were used for validation and resulted in a highly accurate classification of the patients without ovarian cancer (95%), as well as those with benign disease (100%), including all stage I cancers. Overall, this result yielded 100% sensitivity and 95% specificity for the identification of ovarian cancer. The positive predictive value for the sample set was 94%, compared with 35% for the CA-125 serum marker for the same samples. This relatively low positive predictive value can be tolerated when screening a high-risk population. However, as discussed, screening the general population requires a positive predictive value that approaches 100%. Notably, all cases of early-stage ovarian cancer were correctly identified based on their signature proteomic pattern. This observation highlights the potential significance of proteomic technology as a diagnostic tool that will play a role in the discovery of new biomarkers for the early detection of disease [41]. The proteomic pattern that was generated for the ovarian cancer serum samples repre-

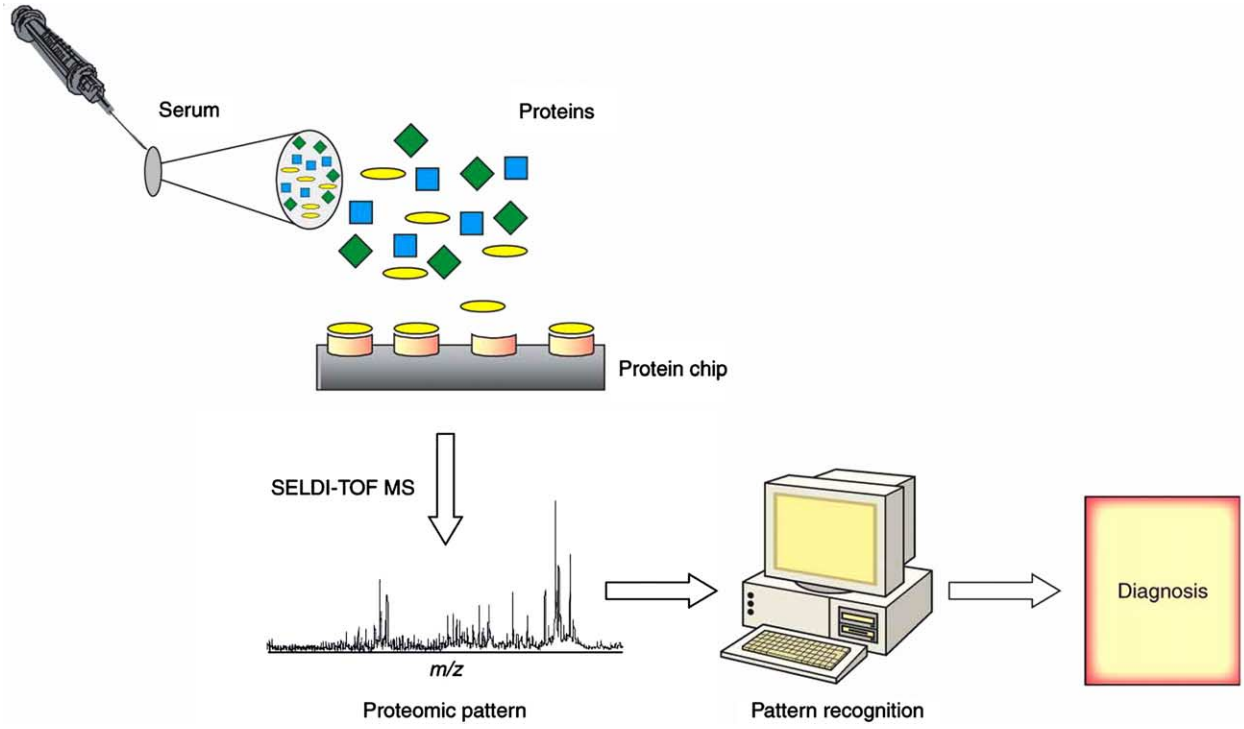

Fig. 1. Disease diagnostics using proteomic patterns. The sample drawn from the patient is applied to a protein chip, which is made up of a specific chromatographic surface. After several washing steps and the application of an energy-absorbing molecule, the species that are retained on the surface of the chip are analysed via mass spectrometry. The pattern of peaks within the spectrum is analysed using sophisticated bioinformatic software to diagnose the source of the biological sample. *m/z*, mass to charge ratio; SELDI-MS: Surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry. [106] Used with permission.

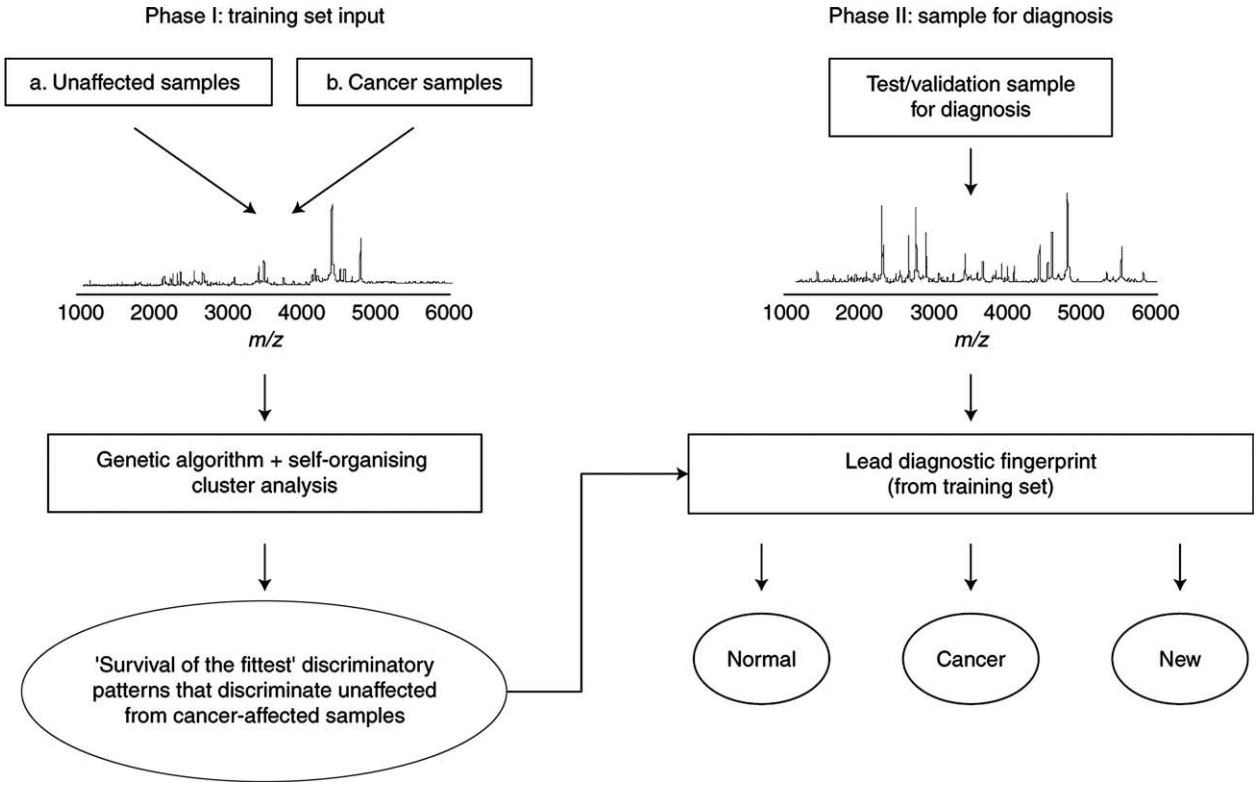

Fig. 2. Bioinformatic analysis of proteomic spectra for the determination of the discriminatory patterns in the training and diagnostic (testing and blind validation) phase [106]. *m/z*, mass to charge ratio. Used with permission.

sents thousands of proteins and peptides that, to date, remain largely unclassified. However, in other proteomic experiments conducted by the NCI–FDA Tissue Proteomic Initiative on microdissected tissue samples of invasive epithelial ovarian cancer and low malignant potential ovarian tumours, differentially and uniquely expressed proteins were characterised between these two ovarian tumour subtypes [42]. The techniques used to accomplish this experiment were a combination of laser capture microdissection (LCM) and two-dimensional gel electrophoresis (2-D PAGE) [42,43]. Three overexpressed proteins in the invasive cancer samples were selected for further analysis and were identified as glyoxalase I, RhoGDI and FK506 binding protein (FK506BP) [42,43]. All of these proteins have been implicated in oncogenesis affected through various pathways, such as tumour cell apoptosis, DNA synthesis and mutagenesis [44,45]. Specifically, FK506BP and RhoGDI are indirect mediators of invasion, which is consistent with the pathological differences between the low malignant potential ovarian tumours and invasive ovarian cancer. Furthermore, using the SELDI method of mass spectrometry, Ye and colleagues reported the identification of the haptoglobin-α subunit in the sera of patients with ovarian cancer for use as a possible serum biomarker for the diagnosis of the disease [46]. They reported a significantly higher peak intensity ($P = 0.002$) in the sera of patients with ovarian cancer compared with controls, which was validated using an enzyme-linked immunosorbent assay (ELISA). Haptoglobin-α subunit may show promise as a serum biomarker used in combination with CA-125 after more studies elucidate the function, as well as the development of a specific antibody assay.

The NCI–FDA studies and others [46,47] demonstrate the feasibility and cost-effectiveness of adding mass spectra analysis not only for diagnosis and the eventual application of this method into routine clinical practice, but also for clinical management of disease such that only a small serum sample is required for analysis [37]. Further clinical trials including large-scale prospective and blinded studies are necessary to validate these initial findings. A future goal is the confirmation of the sensitivity and specificity of this technique as a supplemental tool or an individual approach with regards to the detection of early stage ovarian cancer in the general population.

3.2. The genomic horizon

In general, proteomics represents one of the approaches involved in high-throughput molecular profiling of malignancy and is complemented by genomics. Specifically, DNA microarrays are being used to elucidate the molecular phenotype and gene expression profiles of a number of different cancers, including not

only ovarian, but brain [48,49], breast [50–56], colon [57,58], gastric [59], kidney [60] lung [61–63], prostate [64–67], melanoma [68], small round blue cell tumours of childhood [69] and haematolymphoid system [70–75]. The identification of overexpressed genes or mRNA expression patterns in cancers such as ovarian tumours by cDNA microarray profiling will allow the secreted gene products to be further evaluated as prospective serum biomarkers [76]. Numerous differentially expressed genes in ovarian carcinomas have been reported recently [66,77–84]. However, in terms of their potential usefulness as markers for the early detection of ovarian cancer, the gene product should be secreted. HE4, prostasin, osteopontin, the kallikreins and mesothelin are a sampling of secreted biomarkers that were recognised to be of clinical interest through the use of genomic approaches such as transcriptional profiling.

3.3. The potential genomic markers

The amplification of the HE4 (*WFDC2*) gene has been shown to occur in ovarian carcinomas [66,85]. The initial identification of the *WFDC2* (HE4) was in the epithelial cells of the human epididymis [86], but, subsequently, HE4 overexpression was found in ovarian carcinomas [80,87]. A recent report produced monoclonal antibodies to HE4 epitopes and quantitated the HE4 levels with an ELISA to determine the efficacy of the assay using the sera of postmenopausal women with ovarian cancer compared with matched controls [88]. These blinded studies showed the difference in sensitivity and specificity of the HE4-based ELISA was not statistically significant compared with the CA-125 assay. However, the specificity of HE4 alone was significantly greater ($P = 0.001$) than CA-125 in it is ability to distinguish between malignant and benign disease. This finding provides incentive for future investigations of HE4 as a diagnostic ovarian cancer biomarker to be used in combination with CA-125.

The gene for prostasin, a serine protease, and osteopontin, a bone morphogen, were identified as upregulated by a cDNA microarray assay using RNA from ovarian cancer cell lines and human ovarian surface epithelial (HOSE) cell cultures [28]. This led to an investigation of the secreted gene products in sera of patients with ovarian cancer. Prostasin, which is normally present in prostatic secretions [89], was shown to be significantly differentially expressed ($P < 0.001$) in malignant ovarian epithelial cell lines compared with normal HOSE cell lines by real-time quantitative polymerase chain reaction (RT-PCR) [28]. Validation of prostasin expression with anti-prostasin polyclonal antibodies was performed on surgically removed ovarian tissue, both malignant and benign, and did demonstrate more cytoplasmic immunohistochemical staining in the ovarian cancer cells than the normal ovarian tissue. ELISA was then performed on sera from patients in various stages of ovarian cancer and their levels of prostasin were significantly ($P < 0.001$) higher than in control subjects. Notably, ten patients with stage II ovarian cancer had the highest mean serum levels of prostasin compared with the other stages. This observation is encouraging for the use of prostasin in the early-stage detection of disease. When prostasin and CA-125 serum levels were analysed in combination, it revealed a sensitivity of 92% and a specificity of 94%.

In additional studies performed by the same group, the plasma levels of osteopontin determined by ELISA were reported to be significantly higher ($P < 0.001$) in the preoperative samples from women with ovarian cancer compared with unaffected individuals, who were either without disease, or had benign pelvic disease or other gynaecological malignancies [29]. While larger validation studies are necessary to further evaluate these markers, the relatively low specificity of prostasin and osteopontin when considering them for the detection of early-stage disease at 94% and 80.4%, respectively, suggests that these biomarkers may prove more useful in a screening panel for the detection of preclinical ovarian cancer.

Similar to prostasin, the human kallikreins are secreted serine proteases [90], and one of the most notable kallikreins, hK3 (prostate-specific antigen) [91,92], is already established as a biomarker for prostate cancer. The gene for human kallikrein 10 (hK10) or normal epithelial cell-specific 1 (NES1), known as *KLK10* has been shown to be upregulated in epithelial ovarian cancer [91]. It has been demonstrated that the serum levels of hK10 is significantly elevated ($P < 0.001$) in presurgical samples from patients with ovarian cancer compared with unaffected individuals [93]. The serum levels of hK10 were not significantly elevated in patients with benign gynaecological disease, which confers a greater specificity compared with CA-125 alone. Presurgical and postsurgical levels of hK10 were also evaluated and considerable reductions were found in approximately 70% of the postsurgical specimens [93]. The human kallikrein gene family contains other potential biomarkers, such as hK6 and hK8, which may have a role in the diagnosis, management and prognostication of ovarian cancer when paired with CA-125 [94,95].

Through the use of oligonucleotide and tissue microarrays, mesothelin gene and protein expression were shown to be significantly elevated in serous carcinomas of the ovary compared with other carcinomas and normal tissue [96]. Validation of this expression was performed using immunohistochemistry and more than 50% of cells in 90% of the serous papillary carcinomas of the ovary were reported as positive. In one study, a soluble member of the mesothelin/megakaryocyte potentiating factor (MPF) family was detected in the sera

of patients with advanced stages of ovarian cancer [97]. Further studies are needed in patients with early stage disease to evaluate the use of soluble mesothelin/MPF in screening for ovarian cancer.

4. Other potential markers

Multiple biomarkers have been considered as candidates for the diagnosis of epithelial ovarian carcinomas [2,98]. However, it becomes evident that these lengthy lists may represent promising future directions, but reproducibility and validation are required for definitive qualification. One such example is the marker is lysophosphatidic acid (LPA). There are conflicting reports with regards to the usefulness of detecting significant differences in the serum levels of LPA in patients with ovarian cancer compared with matched controls when using different modalities for detection [99–101].

5. Discussion

In March 2003, The Helene Harris Memorial Trust Forum on Ovarian Cancer discussed the complex and daunting issues regarding the investigation of biomarkers in ovarian cancer such as universal standardisation of guidelines for comparison and validation studies, challenging data analysis as well as sample availability, both before and after diagnosis [102]. The development of screening biomarkers is further hindered by the lack of understanding regarding the clinical biology and progression of epithelial ovarian cancer from preclinical to metastatic disease [2,78]. Hopefully, the information gathered from clinical trials now underway such as the National Cancer Institute's Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening trial [103] and Bart's 3 trial in the United Kingdom [104], will provide some of this much needed information. The development of ovarian cancer biomarkers is just one example of how the burgeoning field of 'translational medicine' not only has the potential to integrate both clinical proteomics and genomics into daily practice, also but to revolutionise medicine [31,105]. Despite all of these exciting advances, further work is needed to be able to effectively screen and diagnose ovarian cancer.

Conflict of interest

None.

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