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European Journal of Cancer 39 (2003) 1818–1827

European Journal of Cancer

www.ejconline.com

Position Paper

Current research and treatment for epithelial ovarian cancer A Position Paper from the Helene Harris Memorial Trust☆,☆☆

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Received 5 June 2003; accepted 5 June 2003

Abstract

In March 2003, an international mulltidisciplinary group of scientists and clinicians with a specific interest in ovarian cancer met for 4 days to discuss research into and treatment of this challenging disease. Under the headings of molecular genetics, molecular biology, the biology of ovarian cancer, old therapies, new targets and the early detection of the disease, this Position Paper summarises the presentations and discussion from the 9th Biennial Helene Harris Memorial Trust Forum on Ovarian Cancer. In particular, we highlight the potential of international collaborations in translating laboratory science into useful clinical interventions.

Keywords: Ovarian cancer; International collaboration; Early detection; New targets; Oeromic profiling; Transcriptional profiling

1. Introduction

Progress in the early detection and improved treatment of ovarian cancer will undoubtedly come from new information concerning molecular genetics, molecular biology and the cell biology of this disease. However, translation of this exciting new information into strategies that will benefit patients requires communication and collaboration between disciplines and investigators. Facilitation of this communication at an international level is the aim of the Helene Harris Memorial Trust (HHMT).

2. Molecular genetics and molecular biology

Global unbiased technologies are being used to analyse genomic [1,2], epigenetic [3], transcriptional profiling [4] and proteomic [5] patterns in tissue and blood samples from patients with benign and malignant ovarian disease. These technologies have great potential for the identification of novel candidates for early diagnosis, indicators of prognosis and response to therapy, and new therapeutic targets.

2.1. Early genetic events in development of ovarian cancer

The rarity of premalignant lesions in epithelial ovarian cancer makes study of early genetic events challenging [6–8]. Age-dependent changes in promoter methylation were found in normal ovarian epithelium (**Baldwin**). 25% of benign tumours and 40% of stage 1 ovarian cancers demonstrated increased methylation of the promoter for the tumour suppressor gene *RASSF1A*, suggesting this may be an early event in ovarian tumori-

☆ Complete abstracts for the meeting and full addresses for the other authors (who chaired the individual sessions) are published on the HHMT website (www.hhmt.org). The names in bold refer to the speakers at the meeting and can also be found on the HHMT website.

☆,☆☆ The Helene Harris Memorial Trust was founded in 1986 by John Harris and his family, in memory of John's wife Helene who died aged 48 years of ovarian cancer. The HHMT promotes and funds dialogue, debate and forums to advance research, diagnosis and treatment in the field of ovarian cancer (www.hhmt.org). The HHMT is collating information for a world-wide database to locate all researchers and clinicians in the field of ovarian cancer. Please send details to database@hhmt.org.

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doi:10.1016/S0959-8049(03)00511-2

genesis. Intriguingly, overall survival was decreased for women with methylation of the *BRCA1* promoter compared with those with *BRCA1* mutations or wild-type *BRCA1* without promoter methylation. The association with prognosis may reflect an effect of global epigenetic aberrations associated with aggressive tumours.

Most micro-dissected epithelial samples from benign ovarian tumours and low-grade ovarian cancers showed allelic imbalance in at least one chromosome arm, affecting chromosomes 5, 6, 7, 9, 11 and 17 in particular (**Campbell**) [9].

Overexpression or mutations of *TP53* were detected in some prophylactic oophorectomy samples, primarily in clefts and inclusion cysts as well as benign tumours (**Boyd**). Where a continuum from normal to malignant ovarian epithelium was observed, *TP53* mutations or p53 immunostaining were found in morphologically normal and dysplastic epithelium. Ovarian cancer may arise from epithelium that has become invaginated into an inclusion cyst (**Boyd**). 189 genes were differentially expressed between cyst and surface epithelium. Many of these were involved in growth regulation and were also altered in ovarian cancers.

Taken together, these data suggest that some invasive ovarian tumours arise from benign epithelial precursors on the surface of the ovary or within inclusion cysts.

2.2. Genomic instability in ovarian cancer

High-resolution comparative genomic hybridisation (CGH) analysis has shown that histological subtypes of ovarian cancer have distinct patterns of genomic change (**Gray**). Moreover, recurrent copy number abnormalities, including increases of 3q, 10q26, 19q12 and 20q12 and decreases of 5q11 and 16q, were strongly associated with a decreased disease-free survival suggesting that these regions harbour genes involved in tumour progression. High-resolution analysis of chromosome 3q demonstrated three separate areas of amplification: one centered at *EVI-1* (ecotropic viral integration site 1), one at *PI3K* (phosphatidylinositol 3′ kinase) and one that was more distal.

Common fragile sites are associated with the presence of large genes (>1 megabase) that are processed into relatively small mRNAs (1–2 kb) (**Smith**) [10]. The large size of these genes, the number of small exons and the degree of processing, may contribute to their sensitivity to DNA damage. Three different fragile sites were characterised in detail, FRA3B, which encodes *FHIT*, FRA16D, which encodes *WWOX*, and FRA6B, which encodes *PARK2*. A novel hypothesis was suggested that fragile sites may play a physiological role in the detection of DNA damage.

2.3. Other tumour suppressor genes in ovarian cancer

WWOX, which codes for an oxidoreductase, is located at the FRA16D common fragile site [11]. *WWOX* is homozygously deleted in a number of ovarian cancers, and in others there is exonic deletion leading to a frameshift (**Gabra**). Enforced expression of *WWOX* markedly decreases *in vivo* growth of ovarian tumour cells, compatible with a putative role in stress responses. Expression of an aberrant *WWOX* isoform may be associated with poor survival in ovarian cancer. *OBCAM* [12], another candidate tumour suppressor gene first identified by loss of heterozygosity (LOH) analysis, is a member of the IgLON family of GPI-anchored cell adhesion molecules. *OBCAM* is frequently somatically inactivated by allele loss or CpG island methylation and occasionally by somatic loss-of-function missense mutations. *OBCAM* displays functional characteristics of a tumour suppressor *in vitro* and *in vivo*.

2.4. BRCA1 and BRCA2

BRCA2-mutant breast cancer cells exhibit high levels of radiation sensitivity [13], as well as selective sensitivity to cisplatin and mitomycin C (**Ashworth**). As cells heterozygous for *BRCA2* do not show increased sensitivity to the effects of radiation, the consequences of radiation would most likely be manifest by cells that have lost function of the normal *BRCA2* allele. Defects in DNA repair could be exploited in BRCA2-mutant tumours, be they breast or ovarian.

2.5. Expression arrays in ovarian cancer

Gene expression array analysis has been used to define the biology of ovarian cancer, to identify correlates of histotypes, to distinguish normal from transformed ovarian epithelial cells and to predict prognosis. Stage I and Stage III high grade serous ovarian cancers exhibit remarkably similar patterns of gene expression, differing quantitatively rather than qualitatively in their profiles [14]. A combination of transcriptional profiling, proteomics and lipomics has identified a number of candidate markers for early detection (**Bast**) [15]. In a histochemical study of 206 ovarian cancers, all tumours could be stained with antibodies against CA125, mucin 1 (MUC1) or vascular endothelial growth factor (VEGF). This suggests that a limited number of markers can detect nearly all ovarian cancers at the tissue level (full listings on genes that are up- or down-regulated in ovarian cancers, from as yet unpublished studies, are available on www.hhmt.org).

2.6. Future directions and initiatives in collaboration

A number of potential early genetic defects in ovarian cancer were described in detail including genomic instability, altered expression of *PI3K, EVI-1*, *WWOX*, *ABCAM, RASSF1A*, altered DNA repair, and aberrant methylation. In the near future, large epidemiological

and expression profiling studies, such as the Australian Ovarian Cancer Study (**Bowtell**), may identify novel molecular subtypes associated with specific epidemiological and genetic risk factors.

Low penetrance genes influencing ovarian cancer may also be identified in large studies of single nucleotide polymorphisms (SNPs) but, in order for studies to be sufficiently powered, international collaboration will be required. In addition, SNPs in genes involved in xenobiotic metabolism may allow individualisation of chemotherapy, e.g. in a study of 57 patients, haplotypes of *CYP3A4* correlated with lack of response to paclitaxel/cisplatin (**Mirhashemi**).

A major resequencing effort aimed at identifying new oncogenes and tumour suppressor genes in cancer has already demonstrated aberrations in BRAF in borderline ovarian cancers and melanomas suggesting that it may be a novel oncogene (**Flanagan**). This has encouraged phase I trials of a novel RAF inhibitor.

Studies of the early steps in tumorigenesis are hindered by the pathophysiology of the disease and a relative lack of high quality samples from individuals with benign lesions or early cancer. The application of global unbiased technologies offers an opportunity to develop profiling data that should subsequently be freely shared with the broader community. We believe that the HHMT could support this process through encouraging the scientific community to share profiling data. The HHMT plans to host a website with links to profiling databases, and to support the development of consortia willing to make tissues or data available.

The NIH Cancer Genetics Network, NIH Breast Cancer Family Registry, NIH SPORE program, the Gynecologic Oncology group, the Women's Health Initiative, and the Australian consortium, kConFab, are collecting patient samples or sera. However, these resources remain limited both in scope and availability and require ongoing support. Furthermore, at this time, changes in patient confidentiality and tissue banking regulations are endangering the ability to utilise these patient resources. Thus, the HHMT should work with academia and Government to ensure that rational regulations are put in place to balance protection of patient confidentiality with efficient research progress.

3. The biology of ovarian cancer

3.1. 'Classical' ovarian cancer xenografts

Over the last 20 years, a wealth of information has been obtained from the implantation of tumour- or ascites-derived human ovarian cancer cell lines into immunocompromised mice. Even today, this system represents the best approximation for the behaviour of the original tumour, including invasion of the peritoneal organs, production of ascites and response to conventional treatments (**Giavazzi**). However, ovarian tumours and cell lines contain numerous genetic lesions thus offering very little information about the tissue of origin and the primary genetic events that trigger tumour formation.

3.2. Transformation of the ovarian surface epithelium in culture

The majority of malignant ovarian tumours are thought to arise from the ovarian surface epithelium (OSE). Unlike rat and mouse OSE which spontaneously transform during prolonged growth in culture [16,17], human OSE (HOSE) cells have a limited life-span of 6–8 passages, and spontaneous immortalisation has not been observed [18]. Transfection of HOSE cells with the simian virus 40 (SV40) early genomic region or with HPV-16 E6/E7 virus can extend life-span in culture, but such lines eventually enter crisis and die.

However, introduction of a combination of genetic perturbations including inactivation of *p53* to *TP53* and *Rb* via SV40 small and large T antigen expression (TAg), and expression of the catalytic subunit of human telomerase reverse transcriptase, was able to immortalise HOSE cells (**Liu**) [19]. Further introduction of either *H-Ras* or *K-Ras* caused tumorigenic conversion of these immortalised cells. When injected into nude mice, the cells developed poorly differentiated carcinomas of the peritoneum with widespread metastasis to the omentum, diaphragm and liver, and massive ascites. Expression array analysis indicated Ras-induced up-regulation of genes required for growth stimulation (interleukin-1 (IL-1)), invasion (matrix metalloproteinase-1 (MMP-1)) and angiogenesis (IL-8), as well as downregulation of collagen type III [20].

3.3. 3.3.Ex vivo *transformation of mouse OSE*

In order to dissect individual biochemical pathways that contribute to ovarian cancer formation, an avian retroviral (RCAS) delivery system was used to introduce defined oncogenes into ovarian cells from transgenic mice bearing the RCAS receptor TVA (**Orsulic**) [21,22]. Ovaries isolated from *p53*-knockout mice, transgenic for TVA under control of the keratin 5 promoter, were transfected with defined oncogenes. Lesions in *TP53* and the addition of any two of the oncogenes *Myc*, K-*Ras* and *Akt* were sufficient to induce transformation. When implanted into the mouse ovarian capsule, the genetically altered cells gave rise to epithelial tumours that resembled human ovarian papillary serous carcinomas and metastasised to the organs that are characteristic of human ovarian tumour spread.

3.4. 'Classical' transgenesis to produce epithelial ovarian cancer prone mice

The first transgenic mouse model of epithelial ovarian cancer was produced by engineering a mouse transgenic for TAg under control of the Müllerian inhibitory substance type II receptor (MISIIR) promoter (**Hamilton**) [23]. OSE is one of several tissue types where the MISIIR promoter is expressed. Approximately 50% of female founder mice bearing the MISIIR-TAg transgene developed tumours by 6–13 weeks of age. Ovaries were almost completely substituted by poorly differentiated neoplastic cells. There was extensive dissemination of the tumour with invasion of the omentum, the mesentery and the parietal and visceral serosa. The tumours were keratin-8- and -19-positive, consistent with an origin from the OSE. The MISIIR-TAg model is currently limited by the early onset of disease and the difficulty of obtaining a stable line in female founder mice.

3.5. Future work in ovarian cancer models

These results support the theory that OSE cells are the precursor tissue for ovarian serous carcinoma. The human OSE model achieves transformation with well-defined genetic changes. However, it is difficult to produce cell lines in which multiple genes are overexpressed and candidate tumour suppressor genes other than *TP53* and *Rb* are deleted. The RCAS system provides the clearest picture of a minimum set of defined genetic perturbations that will yield transformation of the mouse OSE. It allows for simultaneous or sequential introduction of multiple genes without the need for extensive breeding. Perhaps the greatest limitation of this system is that the initiating genetic manipulation is not accurately modelling the sporadic molecular events that occur *in vivo*. However, the genetically defined nature of the model allows for the study of genotype-phenotype correlations.

Disruption of p53 function has been used to drive experimental ovarian oncogenesis in a number of models. As discussed above, there is good evidence to suggest that *TP53* mutations are also an early event in clinical disease.

3.6. Biological studies in human tumours

The progression of ovarian carcinoma is regulated by an interplay of cytokines, chemokines, growth factors and proteolytic enzymes. These factors are especially important in communication between the host and stroma. There is now increased understanding of the key molecules regulating these interactions and this provides another route to developing new therapies. Such novel approaches include anticytokine antibodies, chemokine receptor antagonists, matrix metalloprotease inhibitors and anti-angiogenic factors. These would most likely work when given in combination or in sequence. The relative role of VEGF and matrix metalloproteases (MMP-2 and MMP-9) in progression has been studied in ovarian cancer xenograft models. Blockade of VEGF and MMPs reduces tumour burden and increases mouse survival (**Giavazzi**) [24].

Chemokines are chemoattractant cytokines that play a major role in cell trafficking (**Balkwill**). They are major determinants of the leucocyte infiltrate in cancer and may help establish an immunosuppressive tumour microenvironment [25]. Chemokines also have positive and negative influences on angiogenesis and act as growth and survival factors for tumour and stromal cells.

In ovarian cancer biopsies, expression of the chemokines CCL2 and CCL5, correlated with the extent of the lymphocyte and macrophage infiltrate.

Chemokines may also be involved in the trafficking of tumour cells. Of 14 CC and CXC chemokine receptors investigated, only CXCR4 was expressed on ovarian cancer cells [26]. Its ligand, CXCL12, was found in ovarian cancer ascites and in tumour biopsies, but neither receptor nor ligand was expressed by normal ovarian epithelium. The chemokine, CXCL12, may have multiple biological effects on ovarian cancer cells, stimulating invasion through extracellular matrix, but also facilitating DNA synthesis and establishment of a cytokine network in situations that are sub-optimal for tumour cell growth [27]. Chemokine receptor antagonists are currently being developed for the treatment of inflammatory disease and human immunodeficiency virus (HIV). Preclinical studies are underway to see if specific chemokine receptor antagonists can reduce the leukocyte infiltrate in ovarian cancer or alter the pattern of tumour growth and spread.

One reason why ovarian cancer is a cytokine-driven disease may be because of the production of lysophosphatidic acid (LPA) in tumours (**Mills**) [28]. LPA is a bioactive phospholipid that stimulates cell proliferation, migration and survival. It acts on its cognate G protein-coupled receptors (GPCR), which are ubiquitously expressed and linked to multiple signalling cascades. Aberrant LPA production, receptor expression and signalling may contribute to the pathophysiology of ovarian cancer. The recent identification of ecto-enzymes mediating the production and degradation of LPA, as well as the development of receptor-selective analogues raises the potential for these molecules in cancer therapy.

Many drugs in current use target GPCR receptors making LPA receptors, $LPA_{1,2,3}$, attractive targets. As LPA_1 may be a negative growth regulator, agonists of LPA_1 may decrease the viability and growth of cancer cells. In contrast, LPA_2 and LPA_3, which are over-

expressed in ovarian and other cancer cells, are positive growth and survival regulators for which antagonists may provide potential therapeutic mediators. LPA_3 is particularly appealing as its expression is the most restricted of the LPA receptors and it is aberrantly expressed in multiple cancer lineages.

4. Old therapies and new targets

The use of a platinum drug (usually carboplatin) and a taxane (usually paclitaxel), either in combination or sequence, is now recognised as the 'gold standard' for optimal chemotherapeutic response in women with ovarian cancer.

4.1. New approaches to chemotherapy

A number of novel platinum derivatives have been developed (**Calvert**). The original cisplatin was modified with a cyclobutane bicarboxylic acid replacing two chlorine atoms as the leaving groups (carboplatin), leading to a decreased non-haematological toxicity. Other analogues have been developed that affect the ammine linkage, including oxaliplatin and AMD 473. Oxaliplatin has a 46% response rate in platinum-sensitive disease, 4–14% response rate in platinum-resistant disease and a 20% response rate in taxane-resistant ovarian cancer. AMD 473 produces a 32% response rate in patients with platinum-sensitive disease, but a less than 10% response rate in platinum-resistant patients. As AMD 473 is somewhat more toxic than carboplatin, further trials have not been pursued. Topotecan has been utilised in ovarian cancer using a 5-day schedule. NX211 is a liposomal lurtotecan that permits a 3-day or weekly schedule without a change in toxicity, but with the suggestion that repeated daily dosing may be more efficacious. Epothilone B is a tubulin-binding drug that differs from the taxanes, causing diarrhoea as a dose-limiting toxicity with little neuropathy or myelosuppression. A similar epothilone derivative, BMS 247550, produces more myelotoxicity and moderate neuropathy, but less diarrhoea. Both have exhibited activity near the maximum tolerated dose (MTD) in phase I trials.

Given the relatively small number of patients and large number of promising agents, international collaboration to perform randomised phase II trials would be particularly valuable. Combinations of platinum compounds and paclitaxel with newer cytotoxic agents have been explored in Gynecologic Oncology Group (GOG) pilot studies to establish feasible regimes for phase III evaluation (**Bookman**). In addition, recurrent platinum-resistant ovarian cancer has been a target of phase II studies in the GOG, but few conventional cytotoxic agents have shown promise.

Several novel agents are being evaluated in recurrent ovarian cancer including gefitinib (Iressa), bevacizumab (Avastin), bortezimib (PS-341, Velcade), imatinib (Gleevec, STI-571), polyglutamated paclitaxel (CT-2103, Xyotax), and karenitecin, a lipid-soluble camptothecin. The five-arm GOG0182-ICON5 international phase III trial is based on the incorporation of gemcitabine, topotecan or pegylated (PEG)-liposomal doxorubicin (Doxil) in combination with carboplatin and paclitaxel using doublet and triplet regimens. Accrual has exceeded 1000 patients per year demonstrating the potential of large, multi-armed, cooperative phase III trials to address several concurrent treatment questions. Ongoing phase III trials such as GOG0182-ICON5 involve a substantial commitment of resources over several years, and can become vulnerable to criticism if preliminary data are reported from recently completed studies. Preliminary data can be misleading, and should not generally be used to challenge the ethics or design of ongoing phase III trials. Rather, this information should be used to guide the design of innovative future studies.

There are three randomised trials that suggest a benefit for delivering chemotherapeutic agents intraperitoneally (i.p.) (**Armstrong**). However, the use of this technique has not been widely adopted due to concerns about toxicity and patient acceptability.

4.2. Consolidation and maintenance treatment

Despite modern chemotherapy, most women with advanced ovarian cancer will relapse. Approximately 72% of women who present with distant or metastatic disease will die within 5 years. The development of consolidation or maintenance strategies may improve survival (**Gore**). However, there are few randomised trials in this area and most of these are under-powered. Particular design problems of consolidation and maintenance trials include the timing of randomisation. Consolidation/maintenance trials need to be designed to accommodate trials that investigate new methods of induction, e.g. a 2×2 factorial design may be preferable. A recent trial has shown a progression-free survival benefit for 12 cycles of paclitaxel following standard induction therapy [29].

4.3. Biological and hormone therapy

A number of immunotherapeutic agents have been used in ovarian cancer both systemically and via the i.p. route, and i.p. interleukin-12, in particular, shows promise (**Freedman**). The peritoneum is a specific immunological environment and ovarian cancer is associated with an inflammatory response. Interferon gamma appears to be an active agent in ovarian cancer, particularly when delivered intraperitoneally and this may be

a good i.p. consolidation strategy (**Smyth**) [30]. Recent data suggest that targeting the inflammatory cytokine tumour necrosis factor (TNF) may also have a role [31] and phase I clinical trials of TNF antagonists in ovarian cancer are underway.

Endocrine therapy may have therapeutic potential because many ovarian cancers contained oestrogen (ER) and progesterone receptors (PR). Phase II data suggest activity for tamoxifen and, importantly, it appears to be able to stabilise disease [32]. More selective hormonal agents need to be tested as maintenance therapies in particular, in view of their favourable toxicity profile. The ability to profile ER and PR receptor status, as well as co-activators and co-repressors, should facilitate the selection of appropriate patients in which to evaluate antio-estrogen strategies.

4.4. Signal transduction inhibitors

Tyrosine kinase (TK) inhibitors may have therapeutic potential in ovarian cancer (**Ganesan**). TKs contribute to the development of numerous malignancies including chronic myelogenous leukaemia (bcr-abl), gastrointestinal stromal tumours (kit) and multiple endocrine neoplasia (ret), through constitutive enzyme activity. In the absence of this heightened TK activity, cancer cells die.

Consequently, TK inhibitors have been applied to the treatment of multiple cancers. Small molecule TK inhibitors are available that inhibit epidermal growth factor receptor (EGFR) alone or EGFR in combination with HER-2. Other inhibitors affect VEGFR, platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), KIT, src and TRKA. Criteria for clinical evaluation are being developed to define optimal doses rather than MTDs. Preclinical studies suggest that TK inhibitors will also be active in ovarian cancer, but there are few clinical trials to date. One challenge is to identify those patients most likely to respond based on the expression of activated TKs and other downstream signalling molecules. The experience of Imatinib in CML and GIST is paradigm for signal transduction inhibitors (**Judson**). It appears that the important results obtained in these two tumours with Imatinib may be translatable to other tumour types, but its success in CML and GIST may be related to the fact that there is a single genetic lesion in these tumours [33,34].

4.5. Immunotherapy

Immunotherapy is another form of targeted therapy and there is a need to identify specific targets so that the host response can be optimised by a particular immunotherapeutic manoeuvre (**Freedman**). Expression arrays may be helpful in identifying suitable cellular targets and pathways. Six antibodies or antibody conjugates have been approved by the Food and Drug Administration (FDA) for the treatment of cancer, but none for use against ovarian cancer (**Berek**). In a GOG trial of Herceptin (trastuzumab) less than 12% of ovarian cancers exhibited overexpression of HER-2 and less than 10% of these showed objective responses. The SMART study is evaluating i.p. administration of Theragyn, a ^{90}Y labelled anti-HMFG (MUC1) antibody, in patients without macroscopic disease at second-look laparoscopy. The study has completed accrual, but the data have not yet been analysed. The IMPACT studies utilise Ovarex (oregovemab), a murine monoclonal antibody against CA 125 that acts as a specific immunostimulant. Ovarex complexes with CA 125 antigen in the circulation and is thought to deliver the antigen to dendritic cells, establishing an immune response to CA 125. Subset analysis of phase III data suggest that Ovarex treatment might benefit those patients who respond to chemotherapy and whose CA 125 is less than 65 U/ml after three courses of chemotherapy. Patients who developed an immune response to CA 125 had a survival advantage. A second phase III trial has been initiated in optimally cytoreduced patients who have responded promptly to chemotherapy, comparing Ovarex with a placebo control.

Patients with ovarian cancer have inefficient immune responses (**Disis**). Within solid tumours and ascitic fluid, IL-10 production by monocytes, lack of major histocompatibility components on antigen presenting cells and inhibitory dendritic cells all act together to compromise immunocompetence. In addition, ovarian cancer cells express immunosuppressive TGF-β and evoke CD4+CD25+ immunoregulatory cells. Antigens associated with ovarian cancers are largely 'self' antigens to which cancer patients are tolerant. Levels of specifically immune T cells are generally low when compared with those that develop after exposure to viral and microbial antigens. Combination vaccines that target multiple immunogenic epitopes associated with HER-2 evoke increased numbers of CD4+ and CD8+ T cells capable of recognising HER-2 protein and killing HER-2-positive tumour cells [35]. Endogeneous processing of HER-2 antigens results in 'epitope spreading' of immunity to other HER-2 peptides.

Clinical trials have been performed in HER-2+ve ovarian cancers. Vaccination with HER-2 peptides did not evoke auto-immunity, but 90% patients developed immunity to HER-2 peptides. More than 60% developed a T cell that permitted killing of cancer cells that overexpress HER-2. 70% of patients exhibited epitope spreading. For adoptive immunotherapy, dramatic expansion of T cells that react with HER-2-associated peptides can be achieved *ex vivo* [36,37]. In the future, trials of multipeptide vaccines will attempt to prevent disease relapse. For recurrent disease, a different strat-

egy may be required that includes a combination of vaccines and chemotherapy, manipulation of the microenvironment in the peritoneum, as well as adoptive immunotherapy.

HER-2 overexpression is an important biological factor in the growth of 20–30% of breast cancers and the lessons learnt from the use of antibodies to HER-2 in breast cancer may be relevant to ovarian cancer (**Johnston**). However, as stated above, less than 20% of ovarian cancers show HER-2 overexpression.

4.6. Future perspectives

Emphasis should be placed on more deliberate hypothesis-based evaluation of new agents, particularly in trials sponsored by large cooperative groups. More intelligent use of preclinical and correlative data will be critical for increasing the rate of clinical progress. For each new agent, it will be important to define the characteristics of patients and tumours that are most likely to respond in order to provide a proof of concept. This will require integration of sample collection and analysis into clinical trials. Linking molecular diagnostics with molecular therapeutics will accelerate progress toward individualisation of therapy. Randomised phase II studies can be used to prioritise new agents. In phase I and II studies, optimal biological doses must be found rather than MTDs. Either tumour tissue or surrogate targets, such as the skin or hair follicles, must be analysed, rather than recording only toxicity and activity.

5. Early detection of ovarian cancer

Early detection of ovarian cancer may save lives, but because the disease is relatively rare, screening requires a high specificity. Indeed, specificity of 99.6% is needed to achieve a positive predictive value (PPV) of 10% in postmenopausal women (**Urban**). Because currently available screening tools lack sufficient sensitivity and specificity, efforts are underway to identify better approaches. Any new marker must be highly specific, both to malignancy and to the ovary, and must detect disease as early as possible. It must exhibit stability over time in healthy women to minimise noise relative to signal when used in a longitudinal algorithm.

5.1. Early detection marker discovery

Kallikreins are a subgroup of serine proteases, encoded by 15 genes, localised in tandem on chromosome 19q13.4 (**Diamandis**) [38,39]. Kallikreins (hK5, hK6, hK7, hK8, hK10 and hK11) are elevated or decreased (hK7) in the serum of patients with ovarian carcinoma. For diagnostic purposes, they are not as sensitive as CA 125, but they may be useful in a panel [40]. Investigators in Boston identified three candidate biomarkers for epithelial ovarian cancer by screening RNA from pooled ovarian cancer cell lines and HOSE cell cultures (**Bandera**) [41]. Prostasin, osteopontin, and carcinoma-associated antigen, GA733-2, all displayed a 100-fold increase in the hybridisation signal in cancer cells compared with HOSE. Quantitative real-time polymerase chain reaction (PCR), western blotting and immunohistochemistry validated the overexpression of these genes in micro-dissected ovarian tumour samples. These three markers have the potential to complement CA 125.

Investigators in Seattle identified two promising genes, splice variants of mesothelin [42] and HE4 (WFDC2) [43]. Mesothelin is a differentiation antigen, initially made as a 69-kDa polypeptide. After glycosylation, it is cleaved by a protease to yield a 40-kDa cell bound fragment and a 30-kDa shed fragment. The biological function of mesothelin is unknown, but it may play a role in tumour spread. Soluble mesothelin [44] and HE4 are detectable in the serum of women with ovarian cancer and are complementary to CA 125.

Comparative proteomics facilitates direct discovery of proteomic patterns in the serum (**Kohn and Mills**). Recent advances in technology are yielding proteomic signatures that identify women with ovarian cancer. Investigators in Bethesda are successfully employing surface-enhanced laser desorption and ionisation (SELDI) or matrix-assisted laser desorption and ionisation (MALDI) with time-of-flight detection. These mass spectroscopy approaches allow analysis of the low molecular weight proteome, a heretofore untapped information reserve. A proteomics/bioinformatics approach may have the power to detect ovarian cancer signatures in the statistical range required for a realistic biomarker [5].

5.2. Evaluation of the new markers

Large-scale blinded studies are required to evaluate new markers. Clinical assays must be reproducible and must discriminate between patients with clinically established disease and population controls. Reports should include Risk of Ovarian Cancer (ROC) describing the performance of each novel marker alone and in combination with CA 125. Characteristics of cancers missed by the new marker and CA 125; false-positives, and variability of the marker in healthy women, should all be reported.

Statistical analysis should exploit marker behaviour, such as stability over time in healthy women, and complementarity among sets of markers. Methods for using a single marker in a longitudinal algorithm have been proposed by groups in Boston [45,46] and Seattle [47]. The ROC algorithm proposed by the Boston group is being tested in prospective trials as described below. Methods for combining markers for use in a panel have

been described by the groups in Houston [48] and Seattle [49].

5.3. The need for collaboration

To identify the best set of markers for inclusion in a panel, it is necessary to evaluate several markers in combination in the same set of sera. Samples must be collected, processed and stored identically, because comparability between cases and controls is imperative. Validation studies of this kind are being conducted by at least two groups in the United States (US), both funded by the National Institutes of Health (NIH): the Early Detection Research Network (EDRN) and the Seattle Ovarian Specialized Program of Research Excellence (SPORE). Ultimately, a single prospective study that includes all of the best markers must be conducted, perhaps by investigators brought together by HHMT?

Evaluation of a marker's ability to detect disease requires biomarker measurements in cases prior to diagnosis. Such preclinical samples are very valuable and access to them often requires approval from large investigator committees. Possible uses include validation of early detection marker panels and algorithms, estimation of the duration of the preclinical phase of the disease, and discovery using novel technologies such as proteomics. As the quantity of sample is limited, multiplexing would greatly assist research using these samples. Criteria for access to these samples should be developed by the HHMT investigators for consideration by relevant groups.

5.4. Prospective studies in the final phase of marker evaluation

Once a panel and an algorithm for its use have been defined, prospective screening studies are needed to document that the marker panel detects disease while it is still localised. Rigorous follow-up of screened women yields estimates of the screening strategy's cancer yield and false referral rate. Eventually, studies are needed to directly evaluate the impact of screening on population disease morbidity and mortality, usually in randomised, controlled cancer screening trials.

5.5. Current randomised control trials

The only early detection serum marker that has been investigated in prospective studies is CA 125. Studies are ongoing in two target populations known to be at increased risk—women over the age of 50 years and younger women with a strong family history of ovarian and/or breast cancer. The United Kingdom (UK) Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) will recruit 200 000 postmenopausal women aged 50–74 years. Funded by the Molecular Research Council (MRC)/Cancer Research UK and National Health Service Research and Development (NHS R&D), all 13 regional centres are now actively recruiting and as of May 2003, over 80 000 women have been randomised (**Menon**). In the US, the NIH-funded Prostate, Lung, Colon Ovary (PLCO) Cancer Screening trial involves 74 000 women aged 55–74 years randomised to a screened and control arm. Recruitment is complete and screening is underway.

In women with evidence of a hereditary predisposition, screening is frequently advocated after the age of 35 years, although the efficacy of such surveillance is unknown. The UK Familial Ovarian Cancer Screening Study has begun recruitment, as has a trial in the US under the auspices of the Clinical Genetics Network.

Two pilot trials, European randomised controlled trial of ovarian cancer screening (ERTOCS) and Barts III each involving 13–15 000 average-risk, postmenopausal women, are near completion.

5.6. Novel serum markers have potential application beyond early detection

Mesothelin could also serve as a prognostic marker or even as a therapeutic target in patients with epithelial ovarian cancer, as approximately 70% of these patients have mesothelin-positive tumours [44]. A recombinant immunotoxin, SS1(dsFv)PE38 (SS1P), targeting mesothelin [50] (**Hassan**) has significant antitumour activity against mesothelin-expressing cells, both *in vitro* and *in vivo*, and is currently undergoing phase I evaluation. Prostasin and osteopontin are being evaluated as markers for monitoring disease response.

6. Conclusions

Significant progress is being made in understanding the early genetic events that transform the ovarian surface epithelium, in ovarian cancer biology and the development of rational animal models. Gene expression analysis has already found applications in ovarian cancer and there are some exciting new markers that could be used for early detection, prognostic markers or in the evaluation of therapy. Collaboration is now required in sharing lists of genes that are upregulated in ovarian cancer to accelerate investigation of all potential targets. Studies are underway to identify an optimal panel of markers and mathematical techniques that will identify Stage 1 ovarian cancers. International collaboration will be required for the final assessment of a panel of new markers.

Our meeting also identified an urgent need for international collaboration to develop and implement phase II, consolidation and maintenance trials, to prospectively

collect patient tumours before, during and after therapy and to collect plasma and serum specimens from normal and high-risk individuals.

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