OPINION

Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer

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Abstract | High-grade serous ovarian cancer (HGSOC) accounts for 70–80% of ovarian cancer deaths, and overall survival has not changed significantly for several decades. In this Opinion article, we outline a set of research priorities that we believe will reduce incidence and improve outcomes for women with this disease. This 'roadmap' for HGSOC was determined after extensive discussions at an Ovarian Cancer Action meeting in January 2015.

A recognition of the cellular and molecular diversity of ovarian cancer, and the consequent need for a more refined approach to research and clinical trials, were the key points of a *Nature Reviews Cancer* Opinion article arising from a Helene Harris Memorial Trust (HHMT) Ovarian Cancer Action (OCA) (BOX 1) meeting in 2011 (REF. 1). In contrast to that article, which considered ovarian cancer broadly, here we outline our consensus view on research priorities for a single subtype of ovarian cancer: high-grade serous ovarian cancer (HGSOC). HGSOC is of particular interest, as it accounts for most deaths from ovarian cancer, has shown little improvement in overall survival for decades, and shares substantial molecular similarity with basal-like breast cancer[2]. In addition, our understanding of the molecular aetiology and clinical pathology of HGSOC has greatly increased since 2011, making it important to review priorities in the light of recent research.

Although this disease is termed an ovarian cancer, pathological[3–5], epidemiological[6], molecular genetic[7,8] and mouse model studies[9] suggest that secretory epithelial cells of the distal fallopian tube (FTSECs) are the likely progenitors of a substantial proportion of HGSOCs (FIGS 1,2). However, even with improved methods for pathological assessment of fallopian tubes, some HGSOCs seem to arise without fallopian tube involvement. This is consistent with experimental mouse models of HGSOC: some models show a direct evolution from precursor cells in the fallopian tube[9] and others seem to primarily involve precursor cells in the ovary[10]. It is unclear whether tumours arising without apparent fallopian tube involvement are associated with earlier seeding of the ovaries with FTSECs through a process known as endosalpingosis or whether they are truly ovary-derived diseases[9–11]. Missense or nonsense mutation mutations in *TP53* are currently the earliest known molecular events in HGSOC and a near invariant feature of serous tubal intraepithelial carcinoma (STIC)[12] and HGSOC[13,14] (FIG. 1).

With the exception of *TP53*, *BRCA1* and *BRCA2*, point mutations in oncogenes or tumour suppressor genes are relatively uncommon in HGSOC[14]. Instead, HGSOCs are characterized by genomic structural variation, with frequent DNA gains and losses, making this cancer an extreme example of a chromosomally unstable (C-class) malignancy[15] (FIG. 1). Structural change is an important mechanism for inactivation of tumour suppressors in HGSOC, through heterozygous and homozygous loss[16] and gene breakage[17]. Approximately 50% of HGSOCs are defective in the homologous recombination (HR) DNA repair pathway[14,18,19]. HR defects arise mainly from germline, somatic and epigenetic mutations in *BRCA1* and *BRCA2* (REF. 20) and, to a lesser extent, from mutations in other components of the HR pathway[21] (FIG. 1). HR deficiency is a key determinant of platinum sensitivity in HGSOC and provides a rational basis for the use of poly(ADP-ribose) polymerase (PARP) inhibitors, which further inactivate DNA repair in already compromised HR-defective tumours[22–24].

The molecular characteristics of the other half of all HGSOCs — those that have no apparent defects in HR — are relatively poorly defined. Approximately 30% of this subclass have amplification of *CCNE1* (which encodes the G1/S-specific cyclin E1)[14], and this is likely an early event in the development of HGSOC[25]. Moreover, HGSOC cell lines in which *CCNE1* is amplified undergo cell cycle arrest or apoptosis following the loss of cyclin E1 or its protein partner, cyclin-dependent kinase 2 (CDK2)[26], suggesting a novel therapeutic approach in patients.

Four molecular subtypes (C1/mesenchymal, C2/immune, C4/differentiated and C5/proliferative) have been identified in HGSOC and validated by gene expression profiling[14,27,28]; these are associated with differential clinical outcomes and microenvironmental features, such as immune and stromal cell activation. However, these molecular subtypes have not yet been integrated into the

clinical setting. Recent studies have begun to unravel the determinants of metastatic spread of HGSOC, including its tropism for adipocyte-rich omentum[29] and a propensity for omental localization. Haematogenous peritoneal dissemination has been observed in a parabiosis preclinical mouse model, suggesting that spread of HGSOC throughout the abdomen may occur both passively and via the vasculature[30].

MicroRNA (miRNA) dysregulation has been partially mapped in HGSOC, including the identification of miRNAs that regulate genes associated with the C1/mesenchymal subtype[31–33]. A subset of HGSOCs have shown intratumoural infiltration with activated lymphocytes, in particular $CD8^+$ T cells, and are generally associated with better overall survival. Patients with the C2/immune subtype of HGSOC may benefit from use of immune checkpoint inhibitor therapy[34].

In the light of this new information, at the January 2015 OCA meeting we considered areas of research and clinical practice that we believe will make the most impact on unravelling the molecular biology of HGSOC and developing more effective treatments. This Opinion article outlines seven key areas that we believe offer the most promise in tackling this disease (BOX 2). Supplementary information S1 (box) has a summary of this Opinion article for non-specialist clinicians and the interested public.

Improve current experimental models

Cell lines, patient-derived xenograft (PDX) models and genetically engineered mouse (GEM) models of HGSOC are needed to address different experimental contexts.

Cell lines. Recent studies[35–37] have highlighted the inadequacy of many commonly used ovarian cancer cell lines as models of HGSOC. We strongly recommend that research on HGSOC should use extensively characterized cell lines that accurately reflect the disease, and that their detailed characteristics should be provided in all manuscripts. Development of improved approaches to generate primary cultures from patients[38,39] will assist in producing more effective and accessible models. Collaborative efforts should be directed at creating large sets of genomically and functionally characterized HGSOC cell lines, with clinical annotation and representation of key mutational drivers, such as loss of *BRCA1*, *BRCA2*, *RB1* or *NF1* (neurofibromin 1), amplification of *AKT, PIK3CA* (PI3K catalytic subunit-α), *MYC* or *CCNE1*, and those with a range of nonsense and missense *TP53* mutations. Culture conditions that more closely resemble the tumour microenvironment — including three-dimensional (3D) matrices and co-cultures of malignant cells with fibroblasts and mesothelial cells[40,41] — may also improve success in obtaining continuous, biologically relevant cell lines with stable biologic features. Immortalized FTSECs seem to be the most appropriate normal control for HGSOC[42–44], but further molecular and functional characterization is required of the handful of currently available FTSEC lines[42]. Consideration should be given to generating additional ovarian surface epithelial lines to better understand the role of these cells in HGSOC development.

Mouse models. Considerable progress has been made in recent years in the development of mouse models of ovarian cancer. There are now several GEM models that direct transformation specifically to FTSECs and that histologically and molecularly[9] resemble human HGSOC, including models with mutant *Trp53* and conditional inactivation of *Pten* and *Brca1* or *Brca2* (REFS 8,9) (FIG. 2). Importantly, these models recapitulate the development of the STIC precursor lesion[9,45]. Additional GEM models of other driver events, such as *CCNE1* or *MYCN* amplification, would facilitate studies of other molecular subsets of HGSOC. Novel genome editing technologies may simplify the generation and utility of these new GEM models[46]. Their value will be further enhanced by derivation of transplantable tumour cell lines from these mice with a fully syngeneic background, to allow well-controlled *in vitro* and *in vivo* experiments.

PDXs grown in immunocompromised mice at least partially recapitulate the clinical responses and resistance mechanisms that are observed in patients[47–50]. However, we believe that it is premature to use PDX models derived from specific patients, also known as avatars[51], as a commercial assay to guide drug selection in individual patients. There may be value in exploring humanized mouse models, made by the engraftment of human haematopoietic bone marrow stem cells[52], to overcome the limitations of immune-deficient PDX models. A comprehensive range of HGSOC models is needed to reflect the clonal diversity and the range of acquired resistance mechanisms that have recently been identified[17].

Understanding drug response

Compared with other solid cancers, HGSOCs are unusually sensitive to platinum-based chemotherapy and other DNA-damaging agents, and are frequently amenable to retreatment, even with the same or similar agents to those that were used in the first-line setting. However, treatment resistance eventually emerges in 80–90% of those patients who are initially diagnosed with widespread disease. Genomic studies have shown that substantial clonal diversity exists in patients who have not yet received chemotherapy[53–56], providing a mechanism for the development of resistance. Novel bioinformatic tools that accurately identify and quantify tumour subclones are needed to investigate the evolution of HGSOC genomes under the selective pressure of therapy[57].

In contrast to the existing extensive genomic datasets obtained with primary HGSOC samples, only a handful of recurrent HGSOC samples collected at disease recurrence have been analysed in depth. Consortia such as OCTIPS (Ovarian Cancer Therapy – Innovative Models Prolong Survival) and the British Translational Research Ovarian Cancer Collaborative (BritROC) are focused on obtaining and analysing large intra-patient paired tumour sample sets. Even among the limited number of recurrent samples analysed so far, there is an apparent diversity of acquired resistance mechanisms, including the activation of

Box 1 | **The Helene Harris Memorial Trust meeting on which this article is based**

Ovarian Cancer Action's international research meeting (Helene Harris Memorial Trust (HHMT)), has been fostering communication between international ovarian cancer experts for more than 25 years. With a view to synchronize key ideas and maximize impact in the field, Ovarian Cancer Action brings together the world's leading scientists and clinicians who are dedicated to improving the early detection of ovarian cancers and the treatment of patients with advanced-stage disease (see the Ovarian Cancer Action website for further information). In January 2015, experts met at the HHMT Ovarian Cancer Action 13th International Forum to debate the latest findings in basic, translational and clinical research in high-grade serous ovarian cancer (HGSOC). This article outlines the consensus of the meeting in terms of research priorities, strategies and recommendations for reducing incidence and improving outcomes for women with HGSOC. The listed authors have all contributed to this manuscript.

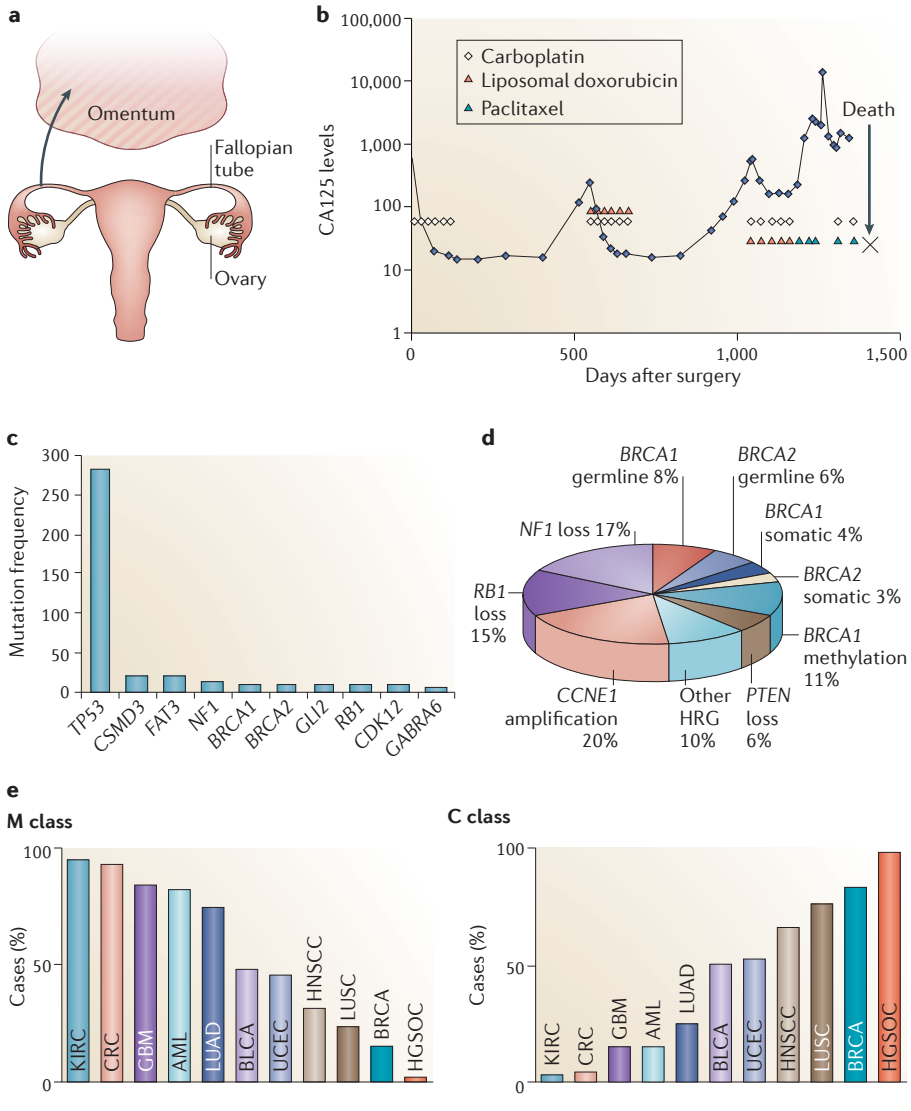

Figure 1 | **Clinical and molecular features of HGSOC at a glance.** **a** | High-grade serous ovarian cancer (HGSOC) is thought to arise predominately from the secretory cells of the fallopian tube, from where there is no barrier to peritoneal spread. HGSOCs have a tropism for omental fat, which they use as an energy source. **b** | HGSOC is characterized by an initial favourable response to platinum-based therapy but then cycles of relapse and the development of acquired resistance to chemotherapy, as depicted by this plot of CA125 levels (in units per millilitre) in a representative patient showing a typical clinical course. Triangles and yellow diamonds indicate administration of different lines of chemotherapy. **c** | *TP53* mutations are a near-invariant feature of HGSOC but somatic point mutations in other driver genes occur at a low frequency. The data shown here were taken from 300 HGSOC tumours in The Cancer Genome Atlas database[14]. **d** | The frequency of key driver mutations in HGSOC, including point mutations, amplifications or gene loss through structural variation (generated from data posted on the cBio Cancer Genomics Portal, Memorial Sloan–Kettering Cancer Center (MSKCC) and REF. 17). Approximately half of all HGSOCs show mutational and functional evidence of putative homologous recombination (HR) deficiency, including germline mutations in *BRCA1* or *BRCA2* in 15–17% of patients. Cyclin E1 (*CCNE1*) amplification represents an important subset of HR-intact tumours, and recent data increase the proportion of tumours with *NF1* (neurofibromin 1) and *RB1* loss. Somatic and germline mutations in components of HR are generally mutually exclusive, as are *CCNE1*, *BRCA1* and *BRCA2* mutations; however, other mutations can co-occur such that individual tumours can have more than one of the driver events represented here. **e** | Graph showing cancer types dominated by either mutations (M class) or copy number changes (C class). HGSOC is one of the most chromosomally structurally variant malignancies. AML, acute myeloid leukaemia; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CRC, colorectal carcinoma; GBM, glioblastoma; HNSCC, head and neck squamous cell carcinoma; HRG, HR-related genes; KIRC, kidney clear-cell carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; UCEC, uterine carcinoma. Part **e** of the figure is from REF. 15, Nature Publishing Group.

AKT signalling[58], the reversion of germline mutations in *BRCA1* and *BRCA2* through intragenic second-site mutations that restore the open reading frame of defective transcripts[17,59,60], the loss of *BRCA1* methylation[17], a shift to a higher stromal content (known as a desmoplastic phenotype) and overexpression of the drug transporter *ABCB1* through promoter hijacking[17]. Targeting of AKT has recently shown promising clinical activity in combination with carboplatin and paclitaxel in a Phase Ib/II study of platinum-resistant ovarian cancer[61]. Expression of markers of autophagy is increased in dormant, drug-resistant tumour nodules found on the peritoneal surface in recurrent disease compared with primary disease[62], suggesting that targeting autophagic processes may be important in overcoming dormancy in HGSOC.

Because only a small number of recurrent tumour samples have been analysed and characterized so far, it is likely that we have underestimated the number of acquired resistance mechanisms. For example, it is unclear whether disease relapse results from the expansion of self-renewing cellular populations, a change in the extracellular matrix, the emergence of drug-resistant clones or a combination of these events, between and within individual patients. We therefore believe that there should be a major effort to characterize recurrent and end-stage samples. Given the importance of understanding resistance, biopsies should be collected at recurrence to generate collections of highly valuable paired pre- and post-treatment samples. Research autopsy studies[17] allow comprehensive sampling to map the diversity of resistance and the collection of large amounts of material for genomic, proteomic, PDX, and immunological and biochemical studies of end-stage disease. Using laparoscopy (minimally invasive surgery for direct visualization of tumours) for tumour mapping, sample collection and prospective monitoring of response to chemotherapy, in particular in the neoadjuvant setting, in eligible patients also offers promise for understanding tumour evolution under primary chemotherapy. Moreover, recent advances in methods of isolating cell-free tumour DNA from patients' plasma samples (liquid biopsies) provide additional, non-invasive means to measure changes in tumours and to understand how cancers evolve in response to treatment[63].

Much of the research on HGSOC focuses on the reasons why some patients have a limited response to chemotherapy. However, it is

also important to characterize the molecular determinants of exceptional responders[64]: those rare patients with extensive post-operative residual disease who have a dramatic and prolonged response to chemotherapy. Exceptional responders may provide insights into the contribution of immunological, stromal or other factors that are important for long-term survival. Comparison of patients with long versus short overall survival may help us to understand how clonal diversity before treatment, genomic instability and the type of host antitumour response influence the emergence of drug resistance. Indeed, a better understanding of host responses to primary and relapsed HGSOC is likely to be central to improving outcomes.

Understand the tumour microenvironment

HGSOC was one of the first human cancers in which an association was found between an increased density of intraepithelial tumour-infiltrating lymphocytes (TILs) and longer patient survival[34]. Tumour-reactive TILs[65] found in HGSOC (FIG. 3) recognize shared tumour antigens such as ERBB2 (also known as HER2), cancer/testis antigen 1 (CTAG1B), mesothelin (MSLN) and telomerase reverse transcriptase (TERT)[66], as well as neoantigens, all processed and presented by major histocompatibility complex (MHC) class I molecules[67]. However, TILs are often suppressed or even functionally exhausted in solid cancers owing to a variety of factors, including: chronic antigen exposure; immune suppressive cytokines (such as interleukin-10 and transforming growth factor-β (TGFβ)); CD47 on tumour cells[68]; metabolite deprivation (including tryptophan depletion by overexpression of indoleamine 2,3-deoxygenase 1 (REF. 69)); immune checkpoint molecules such as programmed cell death protein 1 ligand 1 (PDL1)[70,71]; and the presence of immunosuppressive regulatory T cells and myeloid cells[72] (FIG. 3). Further characterization of the role of immunosuppressive factors in HGSOC is required.

Immune checkpoint inhibition has yielded impressive clinical responses in melanoma and non-small cell lung cancer[73], perhaps owing to the exceptionally high mutational loads in these malignancies[74,75]. By contrast, HGSOC has an intermediate mutational load[14,67,76], and consequently the abundance of neoantigens derived from point mutations is expected to be lower in this disease[76]. TILs are particularly prominent in *BRCA1*-mutant tumours[77–80] for

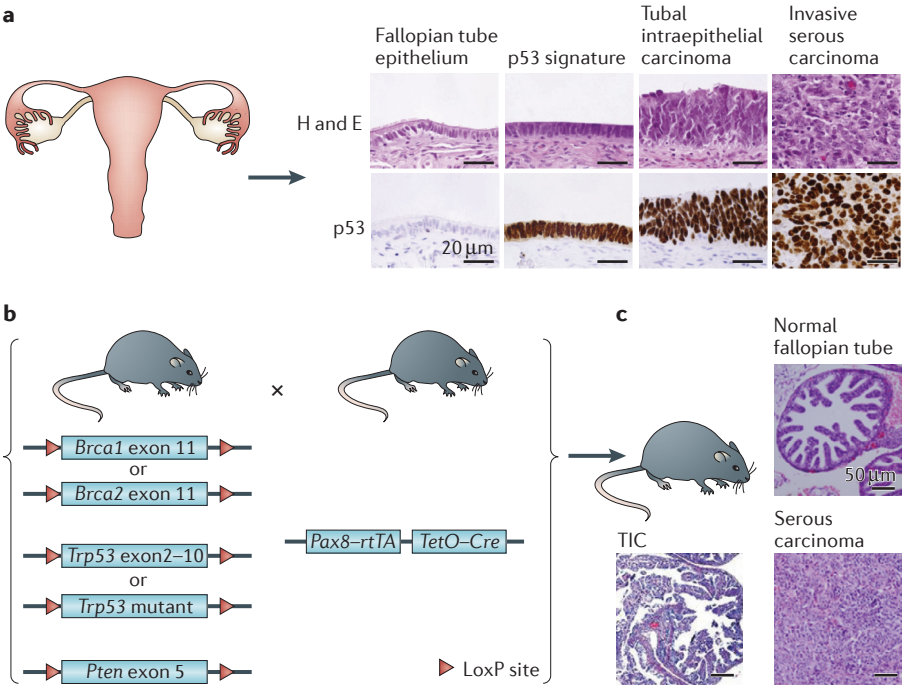

Figure 2 | **Fallopian tube origins of HGSOC.** Animal modelling of high-grade serous ovarian carcinoma (HGSOC) by targeting the fallopian tube and reflecting known mutations in human tumours. **a** | Different stages of HGSOC development in the human fallopian tube marked by p53 staining and cellular morphology. A substantial proportion of HGSOC arises from the fallopian tube, most likely *PAX8*-positive fallopian tube secretory epithelial cells (FTSECs). p53 staining marks clonal expansion of cells (signatures) in the absence of morphological transformation of the fallopian tube epithelium. Piling up of cells and loss of epithelial architecture occurs in early lesions (tubal intraepithelial carcinoma (TIC)), finally leading to invasive cancer. **b** | Crossing strategy to generate a conditional, Cre-recombinase driven model of HGSOC in mice with *Trp53* missense mutation, mutation in *Brca1* or *Brca2*, and dysregulation of the PI3K–PTEN pathway. **c** | The histological appearance of mouse tumours parallels what is seen in human HGSOC. H and E, haematoxylin and eosin; *rtTA*, reverse tetracycline-controlled transactivator; *TetO–Cre*, tetracycline-driven Cre recombinase. Part **a** of the figure is adapted with permission from REF. 184, Elsevier. Figure parts **b** and **c** are adapted with permission from REF. 9, Elsevier.

reasons that are unclear. A prominent TIL response is less commonly associated with *BRCA2*-mutant tumours even though they have more point mutations than tumours with a *BRCA1* mutation[81], indicating that factors other than point mutation load can influence TIL response. The immunogenicity of HGSOC may involve other classes of tumour antigens, such as amplified or aberrant gene products arising from gene fusions. The B cell repertoire is altered in *BRCA1*-mutant carriers[82], suggesting another means by which this germline mutation might affect the tumour microenvironment of HGSOC. An improved understanding of the determinants of TIL density in HGSOC may assist in the development of immune checkpoint therapies in this disease.

Although numerous studies have confirmed the prognostic significance of intraepithelial TILs in HGSOC[27,34], there is currently no consensus on how best to classify immune infiltrates in HGSOC biopsy samples, either for up-front stratification of patients in clinical trials or for post-trial evaluation. With considerable attention now focused on understanding the drivers of the immune repertoire in HGSOC, there is a need to collaboratively develop standard criteria, similar to the 'immunoscore' used to characterize colorectal cancer samples[83]. The development of a well-defined HGSOC immunoscore may require multiplexed immunohistochemistry to capture essential prognostic features, such as immune cell type, functional orientation, location in the tumour and density. Codifying essential elements of an immunoscore would facilitate the comparison of findings from various clinical trials of immune modulators. Patients whose tumours have a high immunoscore may be suitable for immune checkpoint blockade or adoptive T cell

therapies[84], whereas those with an intermediate immunoscore may benefit from agents that stimulate $CD8^+$ T cell trafficking and infiltration. Tumours with a low immunoscore could potentially be treated with vaccines that prime new T cell responses or with engineered T cells, such as chimeric antigen receptor (CAR) T cells that circumvent deficiencies in the tumour-specific T cell repertoire. Studies in lung[85], breast, colon and ovarian[86] cancer cell lines and patient samples show that epigenetic therapies, including DNA demethylating agents[87–89] and some chemotherapeutic agents, can stimulate immune signalling from epithelial cells and may therefore benefit patients with low or absent TILs.

Although TILs are a prominent feature of the tumour microenvironment, their functional phenotype and prognostic effects are strongly influenced by other cell types. The presence of $CD20^+$ B cells in the tumour epithelium[90] and of stromal plasma cells correlate with a better prognosis, whereas regulatory T cells, macrophages and immature myeloid cells may promote tumour formation[91] (FIG. 3). These results are reminiscent of data from colorectal cancer illustrating a common immune phenotype between cancer types[92,93].

Beyond immune cells, the tumour microenvironment of HGSOC has other important elements that may influence treatment response, including fibroblasts, endothelial cells and the extracellular matrix. Recent studies have revealed dynamic interactions between HGSOC and the single cell layer of mesothelial cells that line the peritoneum and pleural cavity[41,94,95], as well as with cancer-associated fibroblasts[96] and adipocytes[29]. These interactions influence tumour spread, metabolism, epithelial–mesenchymal transition and extracellular matrix deposition[96,97], which may in turn affect drug penetration and response to chemotherapy[98,99]. To devise better targeting strategies, it is important to understand whether stromal responses promote or restrain HGSOC[100,101]. Although several Phase III trials have documented the long-term clinical effectiveness of intraperitoneal chemotherapy, the mechanism of action has not been resolved. It is assumed the effectiveness of intraperitoneal chemotherapy is associated with higher drug concentrations in the tumour; however, it is possible that intraperitoneal chemotherapy also alters the interaction of HGSOC with mesothelial cells, which can promote the establishment of metastases in HGSOC[41,94].

Given the profound sensitivity of HGSOC to platinum-based chemotherapy, additional longitudinal studies are required to elucidate the impact of standard treatments on the various cell populations in the tumour microenvironment. Moreover, the complexity of the HGSOC tumour microenvironment means that combination therapies targeting different elements are more likely to be successful than single agent approaches. For example, a current clinical trial combines a Toll-like receptor 8 (TLR8) agonist to activate antigen-presenting cells, liposomal doxorubicin to stimulate immunogenic tumour cell death, and PDL1 blockade to activate T cells (ClinicalTrials.gov identifier NCT02431559).

Stratify patients in trials

The realization that the different types of epithelial ovarian cancer are distinct malignancies has driven the development of histotype-specific clinical trials in recent years. Similarly, an improved understanding of the biology of HGSOC is providing impetus for stratified trials targeting distinct molecular subsets of HGSOC.

Box 2 | Priorities for reducing incidence and improving outcomes in HGSOC

Develop better experimental models
- Develop genomically characterized cell lines and improve methods for growing primary malignant cells and cancer-initiating cells
- Use three-dimensional cultures with other cells in the tumour microenvironment
- Develop patient-derived xenografts that recapitulate clinical responses and resistance
- Develop genetic mouse models that reflect the molecular biology and natural history of the human disease and syngeneic transplantable lines from these

Exploit immune responses and interaction with other tumour microenvironment cells
- Activate suboptimal antitumour immune responses
- Develop an immunoscore for prognostic and therapeutic use
- Study the impact of chemotherapy on the tumour microenvironment
- Understand stromal influences on response to drugs and tumour metabolism

Understand clonal diversity, recurrent disease and exceptional responders
- Analyse recurrent and end-stage disease samples to map acquired resistance mechanisms
- Understand the impact of tumour-initiating cells, resistant clones and changes to the extracellular matrix on relapse
- Characterize clonal heterogeneity and genomic instability in acquired resistance
- Understand the mechanisms of exceptional responses to treatment

Transition to stratified trials of high-grade serous ovarian cancer (HGSOC) subsets
- Molecularly stratified clinical trials based on homologous recombination deficiency, cyclin E1 (*CCNE1*), *AKT1* or *AKT2* amplification, *PTEN* loss and/or molecular subtypes
- Target mechanisms of self-renewal and dormancy
- Evaluate new agents using laparoscopic diagnosis followed by neoadjuvant treatment, interval debulking surgery and measurement of pathological response
- Perform clinical trials of new agents in first relapse of both platinum-resistant and platinum-sensitive disease

Implement strategies that could make a rapid impact on prevention and clinical care
- Highlight the preventive activity of oral contraceptives
- Repurpose drugs with low-toxicity profiles as preventive agents
- Research the value of salpingectomy versus oophorectomy or both
- Effective cascade genetic testing of relatives of affected women and population testing for founder mutations in high-risk groups

Better define the value of surgical cytoreduction
- Research the value of neoadjuvant surgery in advanced-stage disease
- Develop biomarkers to optimize time of surgery for each patient
- Use diagnostic laparoscopy more widely to assess a patient's suitability for surgery
- Revisit 'second-look' surgery to combine with 'window-of-opportunity' trials

Move from 'parts list' to integrated view
- Study the molecular changes in precursor lesions
- Understand the biology of fallopian tube secretory cells and the role of PAX8
- Add metabolomics and proteomic information to genomic and transcriptomic profiles of HGSOC
- Integrate all -omics data on individual samples with immune and other tumour microenvironment components in primary and recurrent samples

HR-defective HGSOC. The pivotal observation of synthetic lethality using PARP inhibitors in *BRCA1*- and *BRCA2*-mutant ovarian cancer cell lines[102] led to the development of one of the most important new classes of targeted agents in HGSOC[23]. Although PARP inhibitors have been most active in patients with either germline or somatic mutations in *BRCA1* or *BRCA2* (REF. 103), significant responses have also been observed in a proportion of non-BRCA-mutant tumours; these tumours might respond because they carry mutations in other genes involved in the HR pathway. The reliable prediction of patient response is an important priority for the development of PARP inhibitors in HGSOC. Although DNA sequencing of all genes involved in the HR pathway is technically feasible, tests that integrate the effects of HR loss, such as functional cellular assays[18] or measuring the genome-wide consequences of defective HR[104] (so-called genomic scarring), may provide a more effective way of identifying the patients who are most likely to respond to PARP inhibitors[105]. Reversion of germline *BRCA1* or *BRCA2* alleles may contribute to clinical drug resistance[17,59,60,106]. Emphasis should be placed on the identification of additional mechanisms of resistance through the analysis of samples collected during disease progression. As has been observed in patients treated conventionally with platinum agents, there are some exceptional responders to PARP inhibitors, and it will be interesting to discover whether the determinants of long-term response to platinum agents and PARP inhibitors are shared.

Anti-angiogenesis. HGSOCs express high levels of pro-angiogenic proteins that contribute to the development of ascites seen in many patients. Therefore, considerable effort has gone into exploring the activity of anti-angiogenic agents in HGSOC, particularly those attenuating the activity of vascular endothelial growth factors (VEGFA and VEGFB). Although the VEGF-specific monoclonal antibody bevacizumab in combination with chemotherapy results in improved progression-free survival in patients in a first-line setting[107,108] and in platinum-resistant recurrent HGSOC[109], its impact has been modest, and there is no evidence of an increase in overall survival[110,111]. Resistance to bevacizumab emerges in most patients with HGSOC who initially respond, but how this occurs remains unclear.

It seems that effective targeting of angiogenesis in this disease is unlikely to be established easily. We therefore recommend that greater effort should be placed on identifying predictive biomarkers, understanding the mechanisms of acquired resistance to angiogenesis inhibitors through the collection of tissue and blood samples from patients enrolled in clinical trials, and the rational development of combination strategies. One recent attempt to identify biomarkers of response to bevacizumab involved transcriptional profiling of samples[112] collected during the ICON7 clinical trial. Surprisingly, patients whose tumour samples had high expression of immune response genes and who received bevacizumab had shorter progression-free and overall survival than patients who did not receive bevacizumab maintenance therapy[112]. If validated, this gene expression profile may provide a useful approach to patient selection and improve our understanding of how different subtypes of HGSOC respond to anti-angiogenic agents. Other molecular agents targeting angiogenesis are in preclinical and clinical development[113,114]. Recent results with the VEGF receptor inhibitor cedarinib and the PARP inhibitor olaparib[115] suggest that combinations of anti-angiogenic agents with other targeted treatments may be beneficial.

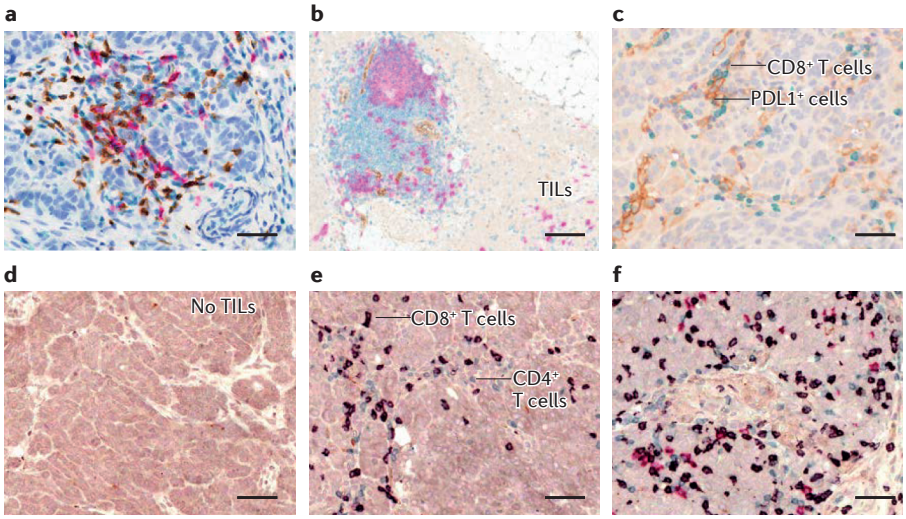

Figure 3 | **The complex tumour microenvironment of HGSOC.** Immunohistochemical staining of high-grade serous ovarian cancer (HGSOC) showing diversity and architectural features of immune cell infiltration. **a** | $CD8^+$ cytotoxic T cell (brown), $CD4^+$ T helper cell (green–blue) and $CD20^+$ B cell infiltration among tumour cells (red). **b** | Tertiary lymphoid structure resembling a lymph node, embedded in tumour, with defined T cell (blue) and B cell (red) zones and associated high endothelial venules (HEVs; brown). Tumour-infiltrating lymphocytes (TILs) are found in the adjacent tumour. **c** | $CD8^+$ T cells (green) are often surrounded by immunosuppressive elements such as programmed cell death protein 1 ligand 1 (PDL1)-expressing macrophages and tumour cells (brown). **d**–**f** | Range of $CD4^+$ (blue–green) and $CD8^+$ (purple) T cell responses in different patient samples in terms of density and association with B cell infiltrate (red). High TIL density (part **f**) is most likely to be associated with therapeutic response to immune checkpoint inhibition. The scale bars represent 100 μm in parts **a** and **c**, 200 μm in part **b** and 50 μm in parts **d**–**f**. Images are courtesy of K. Milne, D. Kroeger and B.H.N. (British Columbia Cancer Agency, Victoria, Canada).

Umbrella trials. The identification of actionable mutations in solid cancers such as lung and breast cancer has led to so-called umbrella trials, in which patients are stratified for treatment according to the molecular properties of their tumours and not the site of cancer origin[116]. Although the limited number of actionable mutations in HGSOC[14] makes the design of umbrella trials challenging, tumours with HR deficiency, amplified genes such as *CCNE1* or *AKT1* and *AKT2* (also known as *PKB* and *PKBB*) or loss of *PTEN* are all suitable for this kind of stratification. The search for novel therapeutic targets should continue by using techniques such as synthetic lethal small hairpin RNA[117,118] and CRISPR (clustered regularly interspaced short palindromic repeats) cell line screens, the high-throughput evaluation of drug combinations[119], and making use of more appropriate HGSOC cell lines and sophisticated culture conditions. Highly multiplexed imaging[120] of the cell surface of HGSOC may provide further novel molecular targets and insights to HGSOC biology.

Although receptor tyrosine kinases (RTKs) are overexpressed in HGSOC, response to single-agent RTK inhibitors has been disappointing[121]. Recent findings

suggest the expression of several RTKs may be deregulated through a novel mechanism associated with methylation-induced loss of expression of OPCML (opioid binding protein/cell adhesion molecule-like), which is a member of the IgLON family of cell surface proteins that modulate receptor recycling[122]. Understanding the pathway associated with OPCML signalling may provide new strategies to revisit the use of RTK inhibitors in HGSOC.

The simple genetic background of other, rarer histological subtypes may also offer the opportunity to study the consequences of some of the less frequent epigenetic and/or mutational alterations found in HGSOC. For example, although missense or deletion mutations in the SWI/SNF ATPase subunit *SMARCA4* (encoding the transcription activator BRG1) are reported in only 2% of HGSOC cases[123,124], this gene is ubiquitously mutated in hypercalcaemic-type small cell carcinoma of the ovary[125]. The self-renewing compartment of HGSOC is only partially defined[126] and may provide insights into new molecular targets for HGSOC. Specifically, molecular characterization of HGSOC stem cells is an important priority for developing maintenance therapeutic approaches that target residual cells following debulking surgery[127–129].

There is a need for clinical trial protocols that allow rapid evaluation of new compounds and combinations of treatments. In breast cancer, window-of-opportunity studies[130], in which new agents are evaluated for a short period of time before surgical resection of the primary cancer, are common. A similar approach could be used in HGSOC, whereby biopsies are carried out at initial diagnosis, followed by several cycles of treatment with a new agent or combination of agents before cytoreductive surgery. This approach would use pre- and post-treatment tumour material to evaluate pathological response as a surrogate endpoint for survival, and such samples could be used for biomarker studies. First relapse provides another setting for testing new agents in HGSOC patients. Although evaluation of new agents at relapse may delay the start of standard treatments, this may be acceptable, as the timing of initiating standard treatment during disease recurrence does not seem to affect outcome[131].

Implement strategies on prevention

Understanding the biology of HGSOC and developing new therapeutic approaches is challenging, complex and time consuming. However, there are already ways to reduce risk and improve clinical outcomes. Oral contraceptives provide lasting risk reduction in both the general female population and in women who carry germline *BRCA1* or *BRCA2* mutations[132], with duration of oral contraceptive use being proportional to the decrease in risk. It is important that women, especially those at increased genetic risk of ovarian cancer, are aware of this benefit. Further research is needed to understand the basis of protection provided by oral contraceptive usage, particularly at a time when intrauterine devices are increasingly favoured for contraception in younger women. Results from two epidemiological studies suggest that chronic use of aspirin to alleviate non-cancer conditions is associated with the unexpected benefit of reducing the incidence of epithelial ovarian cancer[133,134] and that the diabetes drug metformin is associated with improved survival in women with ovarian cancer[135,136]. If these findings are confirmed, repurposing well-established drugs with low toxicity profiles may be valuable in reducing disease burden in high-risk individuals. Conversely, a meta-analysis of 52 epidemiological studies indicates that use of hormonal therapy for menopause moderately increases the risk of ovarian cancer, particularly for serous tumours, most of which are HGSOCs[137]. Obesity also increases the risk of HGSOC[138]. There is an urgent need to understand why there are substantial racial and socioeconomic disparities in the treatment and outcome of women with HGSOC and take steps to close the gap[139].

Germline mutations in *BRCA1* and *BRCA2* are present in 15–18% of patients with HGSOC, and approximately half of all carriers lack a significant family history[20,140–143]. We believe that germline testing should be offered to all women with HGSOC, irrespective of age or family history, with testing done at diagnosis as it provides information about their likely response to therapy[20,103,144]. In addition, there is a case for population testing for founder mutations in *BRCA1* and *BRCA2* in high-risk populations such as Ashkenazi Jewish women, in whom *BRCA1* and *BRCA2* mutations are prevalent. A recent randomized controlled trial involving Ashkenazi Jewish women aged 30 years and older showed that population-based testing could be achieved at an acceptable cost and did not adversely affect short-term psychological and quality-of-life outcomes[145,146]. There is also a need to develop more-effective approaches to 'cascade genetic testing' (REF. 147): predictive genetic testing offered to relatives of index mutation carriers to maximize the opportunities for breast and ovarian cancer risk reduction in female family members and prostate cancer in males.

Less commonly than *BRCA1* or *BRCA2*, patients with HGSOC may have germline mutations in other genes involved in HR, including *BRIP1* (*BRCA1*-interacting protein 1), *BARD1* (*BRCA1*-assoociated RING domain 1), *RAD51B* and *RAD51C*[148–151]. In addition, a series of low risk alleles have been identified through genome-wide association studies[152], but they are currently not clinically actionable. Large cohort studies of population-based cases and controls are needed to understand the penetrance of these mutations — including their interaction with high- and moderate-risk alleles, and the effect of carrying multiple low-risk alleles — and provide useful advice to carriers about their risk of developing ovarian cancer[152]. Clinical panel testing for both high- and moderate-penetrance genes is widely available. Care is needed in counselling unaffected women with moderate-penetrance mutations in terms of ovarian cancer risk-reducing options. In particular, until a better estimate of risk is obtained, we believe that it is premature to offer panel genetic testing beyond *BRCA1* and *BRCA2* as part of routine clinical care for HGSOC. As increasing numbers of women at elevated risk of HGSOC are identified through more comprehensive genetic screening, it will be important to understand the psychosocial[153] and medical needs of women who are at risk but have not yet developed cancer[154].

The improved understanding of the role of the fallopian tube in the genesis of HGSOC has important implications for clinical management. For example, as most hereditary HGSOC are thought to derive from the fallopian tube, removal of only the tubes (salpingectomy) is being considered in young *BRCA1*- or *BRCA2*-mutant carriers to avoid the effects of early menopause that are triggered by removal of the ovaries (oophorectomy)[155]. However, oophorectomy, and the consequent reduction in oestrogen levels, has an added benefit of breast cancer risk reduction in young mutation carriers, and this benefit is absent with salpingectomy only. Hence, there is a need to understand the overall benefits versus side effects of these different approaches in young patients carrying *BRCA1* or *BRCA2* mutations.

Although most HGSOCs may be fallopian tube-derived, there is a subset of HGSOCs with no apparent precursor lesion in the fallopian tube, and so to develop additional prevention strategies we need to understand how these arise. For instance,

further investigation is required of the impact of initial salpingectomy followed by oophorectomy once natural menopause has occurred. Researchers from the British Columbia Cancer Agency are currently investigating the value of removing fallopian tubes in every woman undergoing a hysterectomy, as a practical, population-based, opportunistic approach to reducing ovarian cancer incidence[156].

The identification of increasing numbers of 'at-risk' mutation carriers through wider genetic screening[157] highlights the need for continued efforts to develop an effective screening strategy. The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial using the blood tumour biomarker CA125 and ultrasound showed no reduction in mortality[158]. Recent results from the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) based on serial CA125 profiles seem encouraging[159], although evidence of a mortality impact is awaited. Meanwhile, continued efforts are needed to improve early detection strategies, given the prognostic significance of disease burden at presentation[160]. Research efforts should be based on the understanding of the natural history of HGSOC, with a focus on detection of low-volume disease rather than low-stage disease alone[161]. Future strategies need to incorporate time series algorithms to interpret markers[159,162]. Other approaches include the development of cancer-specific markers such as targeted deep sequencing of DNA for *TP53* and other gene mutations in plasma[163] or cervical secretions[164], and improved[165,166] imaging. Early detection is always going to be a challenge in HGSOC because the disease is often asymptomatic before peritoneal spread.

Define the value of cytoreduction

Pre-operative tumour load and post-operative residual disease are the most important prognosticators of survival in advanced-stage HGSOC[160,167–169]. However, we still do not fully understand which patients are most likely to benefit from primary cytoreductive surgery (or debulking) or how extensive the surgical effort should be[170]. In addition, the timing of surgery remains contentious. The value of neoadjuvant chemotherapy and interval debulking (three cycles of chemotherapy followed by surgery and a further three cycles of chemotherapy) has been established in terms of equivalent overall survival with lower morbidity compared with primary debulking surgery[169]. However, this research was conducted in a setting of limited surgical resection, as evidenced by low rates of optimal cytoreduction and total macroscopic tumour clearance. New trials in specialized centres with experience in maximal surgical effort are needed to re-examine the value of neoadjuvant chemotherapy in advanced disease.

Surgical management of HGSOC should integrate molecular markers with current clinical and pathological factors into an algorithmic approach to surgery[171]. Surgical options include primary or interval debulking or, indeed, no surgery at all in patients with extensive disease and a reliance on chemotherapy alone. The development of biomarkers that reliably predict surgical resectability, or rapid relapse despite optimal surgery, is an important priority for the stratification of patients for these surgical options[172]. The ability to surgically clear all macroscopic tumour tissue (optimal debulking) is influenced by anatomical location and bulk of disease[160], surgical skill, the fitness of the patient to undergo extensive surgery and the intrinsic biology of the epithelial and of other tumour microenvironment components, including TGFβ pathway activation[96,173]. For example C1/mesenchymal molecular subtype tumours have lower rates of optimal debulking and are characterized by a desmoplastic phenotype, which is associated with TGFβ activation[27].

> "seven key areas that we believe offer the most promise in tackling this disease"

The wider use of an initial diagnostic laparoscopy to assess the extent of peritoneal and visceral involvement could assist in triaging patients to primary versus interval debulking surgery while obtaining highly valuable, high-quality research samples[174]. Expanded use of diagnostic laparoscopy may also allow improved prediction of surgical time and the expertise required to achieve optimum debulking surgery. However, no prospective randomized data exist so far to prove the accurate predictive and prognostic value of diagnostic laparoscopy in advanced ovarian cancer, so additional clinical testing is essential.

Although there is no prospective evidence for a survival benefit of secondary cytoreduction after completion of first-line treatment in platinum-sensitive tumours[175], numerous retrospective studies have associated total macroscopic tumour clearance with a significantly prolonged progression-free and overall survival, even at the relapsed setting of the disease[176,177]. Two large, multicentre, prospectively randomized surgical trials (the AGO–OVAR OP.4/AGO DESKTOP OVAR III and the GOG0213) are expected to define for the first time the value of secondary surgical cytoreduction at the time of first platinum-sensitive relapse. Cytoreductive surgery at relapse could also be combined with window-of-opportunity studies.

Move to an integrated view of HGSOC

Genomic analyses, particularly The Cancer Genome Atlas exome, methylome, and transcriptome study of more than 500 primary HGSOC samples, have provided a comprehensive picture of the mutational landscape of primary HGSOC. Although this is an invaluable reference dataset, it falls short of explaining how specific mutations interact to achieve the hallmarks of cancer[178] in an individual patient. Currently, other than understanding that mutation in *TP53* is both an early and invariant event, we know little of the temporal sequence of other molecular changes or the dynamics of chromosomal instability that drive the high degree of genomic aberration in HGSOC. Studies of the molecular changes in precursor lesions, and examination of allelic frequencies of copy number changes and driver mutations, may identify common early, so-called trunk mutations[16]. The centrality of *TP53* mutation for HGSOC suggests that understanding its impact on FTSEC behaviour may provide important insights into initiating events in HGSOC. Indeed, a better understanding of the normal cellular biology of FTSECs as a whole, including growth factor requirements and determinants of self-renewal, is warranted to help interpret how specific mutations and copy number changes affect the behaviour of HGSOC.

PAX8 is a critical determinant of development of the fallopian tube[179] and is expressed[180,181] and required[117] in almost all HGSOCs. Therefore, *PAX8* may also hold clues regarding the molecular circuitry of HGSOC. The tropism of HGSOC for the omentum derives from its propensity to use fat as an energy source[29], which has provided some of the first insights into the metabolic requirements of HGSOC and the determinants of metastatic spread. Metabolomic and proteomic studies, such as the National Cancer Institute Clinical Proteomic Tumor Analysis Consortium (CPTAC)[182], are needed to help interpret current genomic

data and provide insights into post-transcriptional, metabolic[183], signalling and growth factor requirements of HGSOC. Studies that integrate genomic, epigenomic, proteomic, immune and other tumour microenvironment characteristics in a common set of primary and recurrent tumours would be especially informative.

Conclusion

The experimental approaches described here reflect some of the questions that limit the successful management of patients with HGSOC. Only by implementing a more comprehensive and collaborative research approach can we reduce incidence and deliver the long-awaited improvements in survival from a disease that kills an estimated 150,000 women every year.

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Competing interests statement
The authors declare competing interests: see Web version for details.

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