Commentary

Critical Questions in Ovarian Cancer Research and Treatment: Report of an American Association for Cancer Research Special Conference

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Substantial progress has been made in understanding ovarian cancer at the molecular and cellular level. Significant improvement in 5-year survival has been achieved through cytoreductive surgery, combination platinum-based chemotherapy, and more effective treatment of recurrent cancer, and there are now more than 280,000 ovarian cancer survivors in the United States. Despite these advances, long-term survival in late-stage disease has improved little over the last 4 decades. Poor outcomes relate, in part, to late stage at initial diagnosis, intrinsic drug resistance, and the persistence of dormant drug-resistant cancer cells after primary surgery and chemotherapy. Our ability to accelerate progress in the clinic will depend on the ability to answer several critical questions regarding this disease. To assess current answers, an American Association for Cancer Research Special Conference on "Critical Questions in Ovarian Cancer Research and Treatment" was held in Pittsburgh, Pennsylvania, on October 1-3, 2017. Although clinical, translational, and basic investigators conducted much of the discussion, advocates participated in the meeting, and many presentations were directly relevant to patient care, including treatment with poly adenosine diphosphate ribose polymerase (PARP) inhibitors, attempts to improve immunotherapy by overcoming the immune suppressive effects of the microenvironment, and a better understanding of the heterogeneity of the disease. ***Cancer*** **2019;125:1963-1972**. © *2019 American Cancer Society.*

CAN WE DETECT OVARIAN CANCER EARLIER?

Disease limited to the ovary (stage I) can be cured with currently available surgery and chemotherapy in up to 90% of cases, and disease limited to the pelvis (stage II) can be cured in 70% of cases; however, currently only 20% to 25% of patients are diagnosed in these early stages. Computer simulations suggest that detection of a greater fraction of ovarian cancers in early stage could reduce mortality by 15% to 43%.[1,2] The relatively low prevalence of ovarian cancer (1:2500) requires a screening strategy that has high sensitivity (>75%) and extremely high specificity (>99.6%) to achieve a positive predictive value of 10% (ie, 10 operations for each case of ovarian cancer detected). Neither the serum biomarker CA125 nor transvaginal sonography (TVS) used alone can achieve this sensitivity or specificity. A Risk of Ovarian Cancer Algorithm (ROCA) has, however, been developed that measures the trend of CA125 from year to year. Rising CA125 has triggered TVS in 1% to 3% of women screened, and abnormal TVS has prompted laparotomy. Both the Normal Risk Ovarian Cancer Screening Study (NROSS)[3] and the United Kingdom Collaborative Trial of Ovarian Cancer

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Screening (UKCTOCS)[4] conducted in postmenopausal women at average risk demonstrated that, used in this way, CA125 and TVS achieved >99.6% specificity with 3 to 4 operations for each case detected. Both studies detected early stage disease in 40% to 70% of cases. With >200,000 participants, the UKCTOCS was powered to detect a survival advantage. While, overall, the study did not attain statistical significance, a prespecified subset of patients with prevalent disease demonstrated a 20% reduction in mortality ($P < .021$). With wide confidence limits around this estimate, additional follow-up will be required, but clearly there is room for improvement in serum biomarkers and in imaging.

For more effective detection, greater sensitivity is required in the initial phase of 2 stage strategies, while maintaining very high specificity. HE4 and CA72-4 antigens can detect approximately 16% of early stage ovarian cancers missed by CA125 but do not provide lead time. Small amounts of ovarian or fallopian tube cancer can evoke the production of autoantibodies. Virtually all high-grade serous ovarian cancers have mutations of *TP53*, and autoantibodies against TP53 can be detected in 20% of patients, rising a median of 8 months before CA125 and 22 months before diagnosis in patients with normal CA125.[5] HE4 antigen-autoantibody complexes are found in sera from 39% of early stage ovarian cancer patients; CA125 was elevated in 62% and the combination in 80%.

New data indicate that circulating tumor DNA (ctDNA) can be detected in blood or in cervical secretions in 55% of cases with early-stage ovarian cancer complementing CA125 and promising to improve detection, which may be particularly relevant for surveillance of women with BRCA1/2 germline mutations who are delaying preventive bilateral salpingo-oophorectomy to complete their families.[6] In studies of *TP53* ctDNA, preselection of DNA fragments from plasma before assay substantially enhanced sensitivity, and this might also prove useful for detecting amplified or mutant DNA in cervical secretions or uterine washings. Because *TP53* is mutated in a wide spectrum of cancers, determining the tissue of origin for ctDNA assays could prove problematic. Promising data have also been obtained by neural-network analysis of a 9-miRNA panel that can distinguish malignant from benign pelvic masses that include early-stage disease.[7]

With regard to prevention, use of oral contraceptives and pregnancy reduce risk by about 30% each, with greater protection conferred with longer oral contraceptive duration and increasing parity. Recent data suggest that breastfeeding can also decrease the risk of ovarian cancer by about 30%.[8] Longer total duration, increased number of offspring nursed, and earlier age at first breastfeeding increase the protective effect.

HOW DOES THE MICROENVIRONMENT INFLUENCE CANCER GROWTH AND RESISTANCE TO TREATMENT?

Ovarian cancer growth and resistance to treatment are affected by angiogenesis, extracellular matrix, and cancer-associated fibroblasts in the tumor microenvironment. Anti-angiogenic therapy has targeted vascular endothelial growth factor (VEGF) and its receptors (VEGFRs). Most clinical trials with VEGF/VEGFR-targeted drugs have shown improved progression-free survival but not overall survival. Recently, resistance to anti-angiogenic therapy has been associated with the accumulation of tumor-associated macrophages (TAMs). Targeting TAMs using CSF1R-targeted drugs in combination with anti-VEGF drugs has improved outcomes in preclinical models.[9] Another potential target is EGFL6, one of the most highly expressed genes in tumor endothelial cells.[10]

Matrix proteins are dysregulated in ovarian cancer. A comprehensive profile of the ovarian cancer matrisome has been obtained in clinical biopsies by measuring and integrating multiple components, including gene expression, proteomics, cytokine and chemokine levels, cellularity, and extracellular matrix organization. An expression pattern for 22 matrisome genes distinguished patients with a shorter overall survival in high-grade serous ovarian cancer (HGSOC) and 12 other primary solid cancers, suggesting that there may be a common matrix response to human cancer.[11] Networks of cytokines and chemokines appear to regulate the influx of leukocytes into ovarian cancer metastases, and an index of matrisome proteins correlates with infiltration of CD4+ and FOXP3+ T cells.

Cancer-associated fibroblasts (CAFs) from metastatic sites have high expression of nicotinamide *N*-methyltransferase (NNMT). Functionally, NNMT regulates the methylation of repressive histone marks and expression of genes involved in CAF differentiation by depleting *S*-adenosyl methionine levels. Knockdown of NNMT in CAFs was sufficient to attenuate their ability to promote the proliferation, migration, and metastasis of ovarian cancer cells.[12]

DO OVARIAN CANCERS HAVE DISTINCTIVE METABOLIC VULNERABILITIES?

Unlike many other cancers, epithelial ovarian cancer metastases often remain within the abdominal cavity.

Microscopic residual disease (MRD) can persist after primary chemotherapy, grow progressively, and give rise to recurrence, intestinal obstruction, and death. Isolating MRD and measuring genotypic and phenotypic changes in tiny specimens can be challenging. Techniques have been developed to produce whole genome sequencing of picogram quantities of DNA and to measure the prevalence of mutations using phase information.

Within the peritoneal cavity, certain metabolic pathways are key drivers of ovarian cancer cell growth and survival. Ovarian cancer cells can condition CAFs, which, in turn, regulate important cancer cell activities in a paracrine fashion. Adipocytes in the omentum provide fatty acids to adjacent ovarian cancer cells to generate much-needed energy. The fatty acid receptor CD36 is upregulated when ovarian cancer cells are cocultivated with adipocytes.[13]

Metabolism can affect drug resistance. Most ovarian cancer patients receive a combination of carboplatin and paclitaxel, but less than half of patients respond to paclitaxel.[14] Knockdown of the glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 2 (PFKFB2) enhances paclitaxel response in ovarian cancer cells with wild-type *TP53*. Silencing PFKFB2 increases the rate of glycolysis but decreases the flow of intermediates through the pentose-phosphate pathway in cancer cell lines with wtTP53, decreasing NADPH. Reactive oxygen species accumulate and stimulate phosphorylation of Janus kinase, induce G1 cell cycle arrest, and initiate apoptosis that depends on upregulation of p21Cip1 and Puma. Targeting PFKFB2 is a promising strategy for sensitizing ovarian cancers with wild-type TP53 to paclitaxel.

Salt-induced kinase 2 (SIK2) is upregulated in 30% of primary ovarian cancers and overexpressed in omental metastases to a greater extent than in primary cancers.[15] Adipocytes activate SIK2 in ovarian cancer cells leading to downstream phosphorylation of p85 and activation of the PI3K pathway. In addition, SIK2 augments AMPK in regulating fatty acid oxidation and energy production. Targeting SIK2—either genetically or by specific small molecule inhibitors—significantly reduces metastasis in vivo and adipocyte-induced cancer cell proliferation in culture. Knockdown of SIK2 also enhances sensitivity to paclitaxel by inhibiting centrosome splitting and PI3Kinase activity and downregulating survivin.[16] Novel small molecule inhibitors of SIK2-ARN-3236 and ARN3261 enhance paclitaxel response in culture and in xenografts.[17] ARN-3261 will enter first-in-human trials later this year.

Argininosuccinate synthase 1 (ASS1), a crucial enzyme for synthesis of arginine, is lost in a fraction of clear cell ovarian cancers. Because cells with no expression of ASS1 become dependent on external arginine, deprivation of arginine may provide a promising strategy to enhance chemotherapy efficacy.[18]

HOW DO WE MEASURE AND TARGET THE GENETIC, EPIGENETIC, AND TRANSCRIPTIONAL HETEROGENEITY OF DIFFERENT TYPES OF OVARIAN CANCER, COMMON AND RARE?

A significant fraction of HGSOCs are thought to arise from serous tubal intraepithelial carcinomas (STICs) in the fimbriae of fallopian tubes. Although mutation of *TP53* is an early event, loss of heterozygosity for *TP53*, mutations of *BRCA1*/*BRCA2*, loss of *PTEN*, and copy number abnormalities are also found in STICS.[19] Evolutionary analyses reveal that *TP53* signatures and STICs are precursors of metastatic ovarian carcinoma and several years can elapse between development of a STIC and initiation of ovarian carcinoma with metastases, providing an important window of opportunity for early detection and prevention of this disease by surgically removing the fallopian tubes.

HGSOC is typified by frequent copy number alterations across the entire genome with loss of homologous recombination DNA repair machinery. Aside from universal mutation of *TP53* and mutation of *BRCA1* or *BRCA2* in 15% to 20% of cases, HGSOC has a paucity of dominant acting mutations, making targeted therapy directed against driver mutations a difficult strategy to deploy clinically. A recent study has begun to decode the complexity of copy number changes, identifying seven distinct copy number signatures that predict both overall survival and the probability of platinum-resistant relapse. Copy number signatures can also be used to combine agents that are likely to be more effective.[20]

HGSOC cell lines appear to depend on "quality control" pathways that sense and destroy damaged transcripts and proteins. Nonsense-mediated decay (NMD) is highly active in HGSOC cell lines.[21] Interference with enzymes that control NMD either genetically or with small molecule inhibitors is deleterious to HGSOC cell lines. NMD inhibition triggers cell death through activation of the unfolded protein response. Recent studies of HGSOC with multiparametric mass spectrometry have identified rare cell phenotypes within ovarian tumors in addition to the dominant cell subset.[22] Rare populations included ovarian cancer cells that coexpressed

vimentin and E-cadherin, which may play a role in epithelial mesenchymal transition, as well as populations that coexpressed vimentin, HE4, and c-myc, which were associated with poor patient outcome.

Many widely used human and murine ovarian cancer cell lines do not resemble the genotype of HGSOC.[23] Genetically engineered mouse models of HGSOC have been developed in which *TP53*, *BRCA1*, *BRCA2*, *PTEN*, and *NF1* have been deleted in fallopian tube epithelial cells. Although these models have great potential to expand our understanding of HGSOC biology, their use requires large-scale breeding programs, and primary cancers can take many months to develop. Thus, transplantable models remain valuable research tools. ID8, a widely used syngeneic murine model of ovarian cancer, lacks the frequent mutations observed in human HGSOC. Using CRISPR/Cas9 gene editing,[24] novel ID8 sublines have been developed with deletion of *TP53*, *PTEN*, and *NF-1*.[25] Loss of *PTEN* and *NF1* significantly increased the rate of intraperitoneal growth when compared with loss of *TP53*. By contrast, *BRCA1* loss had no effect on intraperitoneal growth, while loss of *BRCA2* actually decreased growth.

A recent review underlines the possible strengths of 1) orthotopic mouse models where cancer cells are injected into the ovarian bursa, 2) patient-derived xenografts (PDXs) where human ovarian cancer cells are grown subcutaneously or intraperitoneally in immune-incompetent nu/nu (T cell deficient), SCID (T and B cell deficient), or NSG (T, B, and natural killer cell deficient) mice, and 3) humanized mice reconstituted with human immunocytes.[26] A majority of PDX models have been shown to correlate histologically, genotypically, and in response to platinum-based chemotherapy; however, with repeated passage, copy number abnormalities have diverged, which is consistent with genetic instability. Use of humanized mice has permitted evaluation of immunotherapy that cannot be evaluated with PDXs in immune-deficient mice, although response to checkpoint inhibitors has been much greater than encountered in the clinic. An important principle to recall is that xenograft models are derived from a single patient and large numbers of different models must be tested to encompass the heterogeneity of clinical cancer.

Low-grade serous carcinomas (LGSOC) account for approximately 10% of all serous cancers and can arise de novo or from serous borderline tumors. LGSOC occur at a younger age than HGSOC and exhibit relative chemoresistance, but are associated with prolonged overall survival. While virtually all LGSOC have wtTP53, up to 40% contain a KRAS mutation and 5-10% contain a BRAF mutation. At least 80% of LGSCs are ER+, and approximately 50% are PR+. In addition, the IGF-1 pathway and angiogenesis appear to be potential therapeutic targets. Clinical benefit of conventional chemotherapy in LGSOC is limited,[27] but bevacizumab,[28] hormonal therapies,[29] and targeted agents, such as MEK or BRAF inhibitors have exhibited greater activity in low-grade serous ovarian cancer than in HGSOC. Currently, second-generation trials of MEK inhibitors[30] are nearing completion, and biomarker studies within these trials should provide important information on the relationship of mutational status and antitumor activity.

Small cell carcinomas of the ovary hypercalcemic type (SCCOHT) are rare but highly aggressive cancers that exhibit truncating or splice site mutations in the SMARCA4 gene that encodes BRG1, 1 of 2 potential ATPases within the SWI/SNF complex. SCCOHT are quite distinctive in that these SMARCA4 mutations are likely initiating events and occur within the context of an extremely quiescent genome. Treatment with EZH2 inhibitors has been proposed and is being tested. Although immune modulation with checkpoint inhibitors have been associated with high mutational burden in other types of cancer, 4 cases of SCCOHT responded impressively to immune modulation therapy, despite a very low mutational burden.[31]

Adult-type granulosa cell tumors (AGCT) account for only 5% of all ovarian cancer and are characterized by a C402G somatic missense mutation in the transcription factor *FOXL2*.[32] Across 3 cohorts, approximately 20% of the cancers thought to be AGCT did not have the pathognomonic mutation and these cancers accounted for 70% of all deaths from disease within the first 5 years. Recently, a TERT C228T promoter mutation was identified in 22% of primary AGCT and 41% of recurrent AGCTs, but not in other histotypes. TERT mutation was significantly more frequent in recurrent tumors ($P = .003$).

Mature cystic teratomas (MCT) are the most common germ cell tumor of the ovary. Less than 1% of MCT transform, usually into a squamous cell carcinoma (SCC), but the mean overall survival of patients with transformed MCT is less than 2 years. In a series of 25 cases, the SCC components had *TP53* abnormalities, *PIK3CA* mutations, and *CDKN2A* abnormalities (deletion or loss-of-function mutation) in 80%, 52%, and 44% of cases, respectively.[33] Cases with mutant *TP53* had a better prognosis than wild-type cases.

CAN WE PREDICT, DETECT, AND REVERSE DRUG RESISTANCE TO CONVENTIONAL AND TARGETED THERAPY?

Targeted therapy can evoke adaptive responses in tissue culture, animal models, and patient samples. Measurement of nodes and pathways that are upregulated by drug treatment can identify effective combinations of targets and drugs. Using reverse-phase protein arrays to identify adaptive responses after treatment with targeted therapy, mutant *RAS* has been found to be a potent mediator of resistance to PARP inhibitors (PARPi) that can be overcome by combinations of PARP and MEK or ERK inhibitors, which could be relevant to treatment of low-grade serous cancers.[34] PARPi induced a STING response that sensitizes syngeneic tumors to immune checkpoint inhibitors, including anti-PD1 and anti-PDL1. BRD4 inhibitors induce marked homologous recombination (HR) defects that synergize with PARPi primarily through the downregulation of CTIP.[35]

MEK inhibition with cobimetinib (GDC-0973) alone had minimal effect on 14 HGSOC PDX models, but produced strong upregulation of the pro-apoptotic protein BIM, which undergoes degradation after ERK activation. Combining targeting of the MEK pathway with inhibition of the anti-apoptotic proteins BCL-2/XL navitoclax (ABT-263) was more effective in reducing cell number and increasing cell death than single agents in the majority of PDX models assessed in vitro and in vivo. Moreover, high pretreatment protein levels of BIM predicted response to combination therapy.[36]

Assay of plasma ctDNA promises to identify relevant targets for therapy. A panel of 508 cancer genes has been used to identify actionable mutations and copy number alterations in patients with HGSOC. Tumor variants could be detected at a low frequency (0.01%) in ctDNA. Altered variants correlated with improved response to treatment. Interestingly, the fraction of tumor-derived variants increased during treatment even in patients with a complete clinical response, potentially detecting subclones that remain refractory to treatment. Some of these variants contain potentially actionable mutations. Interestingly, mutations in chromatin modifiers were significantly enriched among patients with poor response.[37]

HOW CAN WE OPTIMIZE THE ABILITY OF PARPI TO EXPLOIT DEFECTS IN DNA REPAIR?

HGSOC can respond to PARPi, particularly in the presence of *BRCA1* or *BRCA2* mutations or other abnormalities that compromise HR DNA repair. HR dysfunction occurs in nearly 50% of HGSOC.[38] Biallelic loss of *BRCA1*, *BRCA2*, or many of its interacting partners renders cells up to several hundred-fold more sensitive to PARPi. Three PARPi are currently approved for treatment of recurrent ovarian cancer, including olaparib, rucaparib, and niraparib.[39-43] Resistance to these PARPi is a growing problem. A number of mechanisms have been identified, including reversion mutations in *BRCA1* or *BRCA2*, defects in DNA repair caused by loss of REV7 or 53BP1 function,[44] alterations in proteins that stabilize replication forks, and upregulation of PgP transporters. PARP inhibitor resistance can also be caused by mutations in the *PARP1* gene itself. Despite PARP inhibitors resistance, PARP1 mutant tumor cells retain the platinum salt sensitivity seen in *BRCA1* mutant cells, suggesting that cross resistance does not occur.

One rationale for developing combinations of other targeted therapy with PARPi is to convert an HR-proficient cancer into one that is HR-deficient by adding another agent that inhibits HR or replication fork protection such as an anti-angiogenic agent, VEGFR inhibitor, CDK inhibitor, PI3K inhibitor, PD1 blockade, ataxia telangiectasia and Rad3-related (ATR) inhibitor, or CHK1 inhibitor. Trials combining PARPi and these various agents are currently in phases 1 through 3. The oral VEGFR inhibitor cediranib has been combined with olaparib in phase 1-2 trials showing enhanced efficacy in ovarian cancers that were *BRCA* wild-type, suggesting that the addition of an anti-angiogenic agent rendered the cancer cell more HR-like and enhanced the efficacy of the PARPi.[45] Phase 3 prospective trials of this combination are now underway. A phase 1 clinical trial of the PI3K inhibitor BKM120 and olaparib shows clinical activity in BRCA wild-type platinum-resistant ovarian cancer with a 64% rate of benefit, largely from stable disease.[46]

ATR kinase inhibition can overcome acquired resistance to PARPi in *BRCA* mutant cancer cells.[47] *BRCA1* and *BRCA2* control genome integrity through protection of stalled replication forks,[48] in addition to their previously established roles in HR.[49] Interestingly, genetic perturbations that mitigate replication fork stress in *BRCA* mutant cells have been implicated in resistance to PARPi and platinum compounds.[50] ATR is a primary sensor and transducer of replication stress signals, where its kinase activity is induced by events on extended single-stranded DNA segments. Subsequent ATR-dependent phosphorylation and activation of the checkpoint kinase 1 (Chk1) stabilizes replication forks and prevents catastrophic

replication origin firing that leads to genome fragmentation and cell death.[51] Combinations of PARPi and ATR inhibitors have synergistic activity in *BRCA* mutant ovarian cancer cells that had acquired resistance to PARPi.[52]

Chk1 activation is a potential determinant of response to PARPi. Chk1 phosphorylation can be elevated specifically in response to small-molecule PARPi that trap PARP1 on chromatin. In contrast, PARPi that produce minimal PARP1 trapping do not activate Chk1. Chk1 inhibition in combination with a nontrapping PARP inhibitor resulted in improved tumor regression in PDX models of HGSOC. Chk1 phosphorylation may be a convenient biomarker of response to PARPi.[53]

Approximately 20% of HGSOC with amplification of *CCNE1* (encoding cyclin E2), exhibit HR proficiency and de novo resistance to PARPi or platinum compounds. Synthetic lethality has been observed between the presence of high levels of cyclin E and *BRCA* mutation,[54] explaining the mutually exclusive nature of *CCNE1* amplifications and *BRCA* mutations in HGSOC. Rad51 homolog overexpression enhanced transformation of cyclin E–expressing cells. The Rad51 homologs (Rad51 B, C, and D; XRCC2; and XRCC3) are thought to promote canonical Rad51 filament formation and enhance HR and replication fork protection activities.[55] XRCC2 is upregulated in cyclin E–overexpressing cells and is also synthetic lethal in this setting.[56]

DNA damage can induce inflammatory cytokine signaling that might augment effects of immunotherapy.[57] Inflammatory cytokines modify the tumor microenvironment by recruiting immune cells that are critical for both local and systemic responses to immunotherapy and radiotherapy in preclinical murine cancer models.[58] Cell cycle progression through mitosis following ionizing radiation or PARPi is essential to activate type 1 interferon responses. Mitotic progression–dependent inflammatory signaling involves micronuclei formation. Micronuclei frequently rupture in the subsequent interphase,[59] thus exposing genomic DNA to the pattern recognition receptor cGAS. Activation of cGAS within micronuclei signals through the STING protein to promote inflammatory cytokine dependent gene expression. Interestingly, inhibiting progression through mitosis or loss of pattern recognition by cGAS-STING also impairs systemic anti-tumor immune responses in the context of therapy combining ionizing radiation and immune checkpoint blockade. DNA damage–dependent inflammation could be used to harness immune responses that eradicate both chemotherapy-sensitive and -resistant populations.

HOW CAN WE ENHANCE THE IMMUNE RESPONSE IN OVARIAN CANCER?

In contrast to some dramatic results reported in other cancer types, monotherapy with checkpoint inhibitors that bind CTLA4, PD1, or PDL1 has produced response rates of <15% in unselected ovarian cancer patients. Use of checkpoint inhibitors individually or in combination can induce substantial toxicity from autoimmune disease. There is clearly a need to identify biomarkers for response or lack of response to these agents. Based on infiltration of CD8, T regulatory, and B cells into the epithelial and stromal compartments, ovarian cancers can be immunologically "cold," "warm," or "hot." High levels of CD8 TIL are associated with a favorable prognosis but have not yet been shown to predict a response to PD1/PDL1-targeted immunotherapy, consistent with the presence of additional immunosuppressive factors in the tumor microenvironment.

Better understanding of immunologically inert or "cold" tumors may represent an attractive therapeutic opportunity, because they can express high levels of tumor-specific antigens with corresponding systemic T cell and antibody responses. After neoadjuvant chemotherapy, 1) TIL^{high} tumors showed increases in multiple immune markers; 2) TIL^{low} tumors underwent similar increases, achieving patterns indistinguishable from the first group; and 3) $TIL^{negative}$ cases generally remained negative.[60] Tumor deposits with a high degree of clonal heterogeneity generally have low densities of immune infiltrates, suggesting a means by which tumor evolutionary processes are insulated from immunologic attack.[61] T cell clones track with individual tumor clones across space, suggesting that the immune system contends with intratumoral heterogeneity by battling each tumor clone individually. In particular, observations of clonally diverse primary foci present in conjunction with distal clonally pure sites could indicate local immune privilege at sites with divergent clones and active immuno-selection at more clonally pure sites. This work is providing novel insights into the relationship between the clonal architecture of tumors and antitumor immunity. One route to deliver T cell immunotherapy to ovarian cancer patients is to enrich naturally occurring tumor-reactive T cells from "hot" tumors and expand them to large numbers for autologous infusion. Methods have now been developed to enrich and expand tumor-reactive tumor infiltrating lymphocytes (TIL) and to eliminate nonreactive bystander cell subsets. The subset of ovarian cancer TILs with potent antitumor activity expresses the CD137 molecule and can be enriched through

magnetic sorting. This tumor-reactive TIL fraction expands in an HLA-dependent manner in the presence of interleukin (IL)-7 and IL-15, but not IL-2. Antigen presenting cells that are genetically modified to express costimulatory ligands for T cells (eg, CD137L) can expand these tumor-reactive ovarian cancer TILs to levels greater than those seen in standard IL-2 culture conditions. On the basis of these and other findings, several clinical trials of TIL therapy for ovarian cancer are ongoing.

Multiple approaches are being tested to overcome the immunosuppression observed in ovarian cancers. Indoleamine-2,3-dioxygenase (IDO) 1 expression depletes tryptophan and enhances synthesis of immunosuppressive metabolites that can decrease the activity of checkpoint inhibitors in ovarian and other solid cancers.[62] Administration of the IDO inhibitor INCB024360 before surgical resection of ovarian cancer reduced IDO enzyme activity, increased $CD8^+$ T cell infiltration, and reduced suppressive T regulatory cells. A second strategy to reprogram the immunosuppressive ovarian cancer microenvironment utilizes the repeated intraperitoneal administration of a human IL-12 DNA expression vector within a synthetic polyethyleneglycol–polyethyleneimine–cholesterol delivery system during neoadjuvant chemotherapy before interval cytoreduction.[63] Intraperitoneal IL-12 gene therapy led to a preferential increase in IL-12 and interferon-γ levels in the peritoneal cavities of patients, a decrease in T regulatory cells, an increase in $CD8^+$/ratio in 60% to 80% of patients, and a shift from naive $CD8^+$ cells to effector memory cells. A third approach to overcoming immunosuppression is to engineer T cells that not only express the T cell receptor specific for NY-ESO-1, but also a decoy receptor that renders the T cells resistant to immunosuppression by transforming growth factor β. A phase 1/2 clinical trial testing this approach is currently open and accruing.

For patients with immunologically "cold" ovarian cancers, lysates generated from the patient's own tumor have been used to vaccinate against shared antigens as well as patient-specific mutated antigens.[64] In this prime-and-boost approach, a patient can first be vaccinated against patient-specific antigens to induce antitumor immunity, and the vaccine-primed T cells are then harvested and expanded to high numbers outside of the body before reinfusion into the patient. The feasibility and safety of this approach has now been established in ovarian cancer with evidence of biologic activity. Particularly promising results have been obtained using personalized vaccines generated by pulsing autologous dendritic cells (DCs) with oxidized autologous whole tumor cell lysate, which was injected intranodally in platinum-treated, immunotherapy-naive, recurrent ovarian cancer patients alone or with bevacizumab with or without low-dose cyclophosphamide.[65] Vaccination induced T cell responses to autologous tumor antigen, which were associated with significantly prolonged survival. Vaccination also amplified T cell responses against mutated neo-epitopes derived from nonsynonymous somatic tumor mutations, which included priming of T cells against previously unrecognized neoepitopes, as well as novel T cell clones of markedly higher avidity against previously recognized neoepitopes.

Inhibitors of DNA methyl transferase 1 (DNMTI) can enhance expression and presentation of tumor antigens that can be recognized by the adaptive immune system, including "cancer testis antigens" such as NY-ESO-1. Adoptive transfer of HLA-A*02 restricted clones of NY-ESO-1–specific CD8 TCR gene-engineered T cells in combination with the demethylating agents decitabine and SGI-110 elicited synergistic inhibition of tumor growth, curing a fraction of mice. In the NY-ESO-1–negative OVCAR3 model, demethylating agents not only induced expression of NY-ESO-1 tumor antigen and major histocompatibility complex I and II, rendering the tumor visible for recognition by CD8 T cells, but also dramatically promoted persistence and accumulation of adoptively transferred T cells at the tumor site, as well as reduction of suppressive myeloid cells in the tumor.[66] DNMTI rendered the tumor visible for recognition by NY-ESO-1–specific CD4 T cells, leading to significant tumor inhibition, and improved the persistence of CD4 T cells at peripheral and tumor sites.

MUC16, the glycoprotein encoding the CA125 antigen, can function as an oncogene. The carboxy-terminal portion of the MUC16/CA125 protein transforms NIH/3T3 cells, increases invasive tumor properties, activates the AKT and ERK pathways, and contributes to the biologic properties of ovarian cancer. The MUC16 oncogenic effects are mediated through *N*-glycosylation of asparagine sites within the 58–amino acid domain between the putative cleavage site and the cell membrane. Oncogenic signaling requires the presence of galectin-3 and growth factor receptors colocalized on lipid rafts. With sufficient *N*-glycosylation and galectin-3, MUC16 stabilizes progrowth receptors on the cancer cell surface and enhances signaling through decreased receptor turnover. Monoclonal antibodies that

block galectin-3–mediated MUC16 interactions with cell surface signaling molecules inhibit invasion of ovarian cancer cells, directly blocking the in vivo growth of MUC16-bearing ovarian cancer xenografts, providing a new therapeutic approach. MUC16-targeted chimeric antigen receptor (CAR) T cells directed at the most proximal portions of MUC16 have been developed and are currently being evaluated in clinical trials.[67]

CONCLUSIONS

In answering these "critical questions," we have learned that screening algorithms measuring the trend of CA125 values over time can achieve adequate specificity, but we must improve the sensitivity of panels of biomarkers for early detection of ovarian cancer, possibly utilizing autoantibodies, antigen-autoantibody complexes, and nucleic acids. Macrophages in the tumor microenvironment can increase resistance to anti-angiogenic therapy, and cytokines, chemokines, and matrix proteins can influence the influx of immunoregulatory cells. Ovarian cancer cells exhibit distinctive metabolic changes that can be targeted, including a dependence on fatty acids from adipocytes, aberrant glycolytic pathways, overexpression of SIK2, and dependence on arginine in different histotypes. Heterogeneity is observed between and within ovarian cancers of the same histotype. New targets have been identified in rare histotypes, and new technologies have identified diverse and potentially important subpopulations within high-grade serous ovarian cancers. There is still a critical need to develop more predictive animal models, as well as to test new agents and approaches in multiple models as each may reflect the genotype and phenotype of only a single patient. Targeting pathways upregulated by individual drugs can overcome adaptive resistance. Three different PARP inhibitors have been approved for treatment of women with ovarian cancer and combinations of PARP inhibitors with PI3K inhibitors, MEK inhibitors, ATR inhibitors and CHK1 inhibitors are being evaluated to overcome PARP inhibitor resistance. Finally, immunotherapy with immune checkpoint inhibitors has produced only a 10% to 15% response rate in ovarian cancers, which is related, in part, to the heterogeneity of immune infiltrates in tumor tissue. Novel approaches are being developed to overcome the immunosuppressive microenvironment of ovarian cancers, to present autologous tumor-associated antigens more effectively, and to administer genetically engineered CAR T cells. These advances promise further improvement in patient outcomes over the next years.

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CONFLICT OF INTEREST DISCLOSURES

Robert C. Bast Jr receives royalties for the discovery of CA 125 from Fujinabio Diagnostics Inc. Ursula A. Matulonis has acted as a member of the advisory boards for AstraZeneca, Myriad Genetics, Clovis, Eli Lilly, Mersana, Geneos, Fuji Film, and Cerulean and as a paid consultant for Merck, 2X Oncology, and Immunogen for work performed outside of the current study. Anil K. Sood has acted as a member of the scientific advisory board for Kiyatec, has received a grant from M-Trap, and is a shareholder in Biopath for work performed outside of the current study. Ahmed A. Ahmed has a patent pending for biomarkers for the early diagnosis of ovarian cancer. Monicka Wielgos-Bonvallet has received a grant from the National Center for Advancing Translational Sciences of the National Institutes of Health for work performed as part of the current study. James D. Brenton has acted as a cofounder and consultant for Inivata Ltd, has received funding to University of Cambridge for academic research collaboration grants from Aprea, has received nonfinancial support from Clovis Oncology, and has received personal fees from Bayer and AstraZeneca that were paid to the University of Cambridge for work performed outside of the current study. Joan S. Brugge has received honorarium for acting as a member of the scientific advisory board from Agios Pharmaceuticals, has received honorarium for acting as a member of the scientific advisory board from and holds stock options in Effector Pharmaceuticals, and has received grants from Roche Pharmaceuticals for work performed outside of the current study. Robert L. Coleman has received grants from the National Institutes of Health (2P50 CA109298 and P30CA016672), Gateway Foundation, and VFounation for work performed as part of the current study and has received grants from and acted as a paid consultant for AstraZeneca; has received grants from Merck; has acted as a paid consultant for Tesaro and Medivation; has received grants from and acted as a paid consultant for Clovis; has acted as a paid consultant for Gamamab; has received grants from and acted as a paid consultant for Genmab, Roche/Genentech, and Janssen; and has acted as a paid consultant for Agenus, Regeneron, and OncoQuest for work performed outside of the current study. Giulio F. Draetta reports personal fees from and stock ownership in Karyopharm Therapeutics and Metabomed, personal fees from Blueprint Medicines and Taiho Pharmaceuticals, personal fees from and stock ownership in BiovelocITA, personal fees from Helsinn Ventures, and personal fees from and stock ownership in Forma Therapeutics and Orionis Biosciences and Nurix Inc for work performed outside of the current study. Ronny I. Drapkin has acted as a member of the scientific advisory board for Repare Therapeutics, as an ad hoc advisor for nVision Medical, and as a member of the scientific advisory board for Pear Tree Pharmaceuticals and Siamab Therapeutics for work performed outside of the current study. Mark A. Eckert has a patent for droplet microfluidics for single cell analyses with royalties paid and a patent for engineered stem cell therapies for cancer with royalties paid. Kevin M. Elias has received grants from NICHD Reproductive Scientist Development Program (K12HD000849), the Robert and Deborah First Family Fund, the Honorable Tina

Brozman Foundation, the Minnesota Ovarian Cancer Alliance, and the Saltonstall Foundation for work performed as part of the current study and has a patent WO2018129535A1 pending relating to the use of circulating microRNAs for the diagnosis of ovarian cancer. David M. Gershenson reports an equity interest in Biogen Inc, Johnson & Johnson, and Celgene, and has acted as a member of the advisory board for Clovis Oncology for work performed outside of the current study. Ernst R. Lengyel has a patent CT 45 in cancer pending and a patent FABP4 in ovarian cancer issued. Christopher J. Lord reports grants from AstraZeneca, Merck KGaA, and Artios for work performed as part of the current study; has acted as member of the scientific advisory board for AstraZeneca, Merck KGaA, Artios, and SunPharma; has acted as a paid consultant for GLG; has acted as member of the scientific advisory board for Vertex; has acted as a paid consultant for and owns stock in Tango; and has acted as a paid consultant for OnoPharma for work performed outside of the current study. In addition, Dr. Lord is a named inventor on patents describing the use of DNA repair inhibitors and stands to gain from the development as part of the ICR "Rewards to Inventors" scheme. Gordon B. Mills has received grants from the Susan G. Komen Breast Cancer Foundation, Ovarian Cancer Research Foundation, Breast Cancer Research Foundation, Adelson Medical Research Foundation, and Pfizer Pharmaceuticals; has received grants and personal fees from and acted as a member of the scientific advisory board for AstraZeneca; has acted as a paid consultant/member of the scientific advisory board for and has stock/options/financial in Catena Pharmaceuticals; has acted as a paid consultant/member of the scientific advisory board for and received grants from Critical Outcome Technologies; has acted as a paid consultant/member of the scientific advisory board for, has stock options in, and has received sponsored research from ImmunoMET; has acted as a paid consultant/member of the scientific advisory board for and has received sponsored research from Ionis; has acted as a paid consultant/member of the scientific advisory board for and has stock/options/financial in Signalchem Lifesciences; has acted as a paid consultant/member of the scientific advisory board for Symphogen; has acted as a paid consultant/member of the scientific advisory board for and has received sponsored research from Takeda/Millenium Pharmaceuticals; has acted as a paid consultant/member of the scientific advisory board for and has stock options in Tarveda; has stock options in Spindletop Ventures; and has received sponsored research Karus Therapeutics, Nanostring, and Prospect Creek Foundation for work performed outside of the current study. In addition, Dr. Mills has a patent HRD Assay to Myriad Genetics licensed to Myriad Genetics, and a patent DSP patent to Nanostring licensed to Nanostring. Brad H. Nelson has received personal fees from Merck, SymVivo, ImmunoVaccine, and Qu Biologics and a research grant and in-kind research funding grants from Zymeworks for work performed outside of the current study. Daniel J. Powell Jr has a patent US20160215262A1 issued. David R. Spriggs has received a grant from the National Cancer Institute (1 P01 CA190174) for work performed as part of the current study. In addition, Dr. Spriggs has a patent for MUC16 directed antibodies issued to MSK, licensed to Juno Therapeutics. The other authors made no disclosures.

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