

Therapeutic Innovations in Ovarian Cancer Treatment: The New England Perspective

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Abstract

Ovarian cancer accounts for more deaths in the United States than all other gynecologic malignancies combined. More than two-thirds of patients are still diagnosed, as they always have been, with advanced stage disease. Traditionally, upfront surgery followed by platinum-based chemotherapy has been the treatment of choice. More recently, the paradigm of care has undergone significant revision as our understanding of this heterogeneous disease continues to evolve. As a result, the way we think about, categorize and approach, the treatment of ovarian cancer is changing rapidly. Increasingly, innovations in targeted therapies appear to be the future. The purpose of this review is to provide an update of current controversies in the field, and to highlight new directions in the management of this insidious disease.

Keywords: Ovarian cancer; Primary debulking surgery; Therapeutics

Introduction

Ovarian cancer was once described, not so long ago, as the 'silent killer' due its nonspecific symptoms, and sudden appearance of advanced disease. Subsequently, a more accurate portrait has been demonstrated, wherein patients frequently experience bloating, urinary frequency, abdominal pains, or early satiety on a regular basis [1]. The majority will have seen a health care provider, often multiple times, without having a pelvic examination performed, but leading to further delays in diagnosis [2]. Unfortunately, neither physician education, nor patient recognition of symptoms has led to improvements in early detection [3]. Moreover, despite enormous expense and thousands of patients under study, no serum marker, panel, or screening imaging test appears to be emerging as a practical method of decreasing mortality [4]. In fact, the US Preventative Task Force specifically recommended against routine screening of the general population in 2012 [5]. Due to the very limited success at achieving an earlier diagnosis, two-thirds of women developing ovarian cancer will have advanced disease at the time of diagnosis.

Primary debulking surgery (PDS) was initially shown to have clinical benefit in the 1970s, and dozens of retrospective studies in the 1980s and 1990s appeared to confirm its central role [6-8]. Postoperatively, the combination of intravenous paclitaxel and a platinum drug emerged as the standard treatment [9]. For the most part, all patients with epithelial ovarian cancer were shoehorned into this simple two-step schema and this paradigm became entrenched within the medical community. Unfortunately, relapse rates were high, outcomes often grim, and limited progress was made until the traditional dogma was challenged.

Recently, a number of exciting developments have occurred, especially within the last decade. With a view toward reducing morbidity and establishing a better system to triage only the best candidates for PDS, minimally invasive procedures may have an expanding role. For high-risk or unresectable patients, neoadjuvant chemotherapy (NACT) has not been shown to be an inferior strategy, and the potential for longitudinal tissue collection allows for the

inclusion of innovative translational study endpoints in clinical trials. The value of intraperitoneal (IP) chemotherapy remains a controversial topic, and one that continues to be rigorously studied. Due to the high risk of relapse from advanced ovarian cancer, maintenance therapy has been a popular, but thus far unproven modality for prolonging remission. Rare tumor types, once at the periphery, and unable to be studied within a prospective trial due to logistical reasons, are now the focus of medical breakthroughs. Genetic testing is occupying more of a role, not just for counseling family members, but to identify candidates for targeted therapy. Development of novel agents specifically directed at the chemo-resistant pluripotent ovarian stem cells is underway, as are innovative immunotherapeutic drugs.

We are witnessing dramatic changes in the way we think about, categorize and approach the treatment of ovarian cancer. Distinct molecular alterations are helping to explain why this disease which can look so similar histologically, has such a heterogeneous response to therapy. The purpose of this review is to provide an update of current controversies in the field, and to highlight new directions in the management of this insidious disease.

Minimally invasive surgery

One of the conundrums of primary debulking surgery (PDS) is that it is only beneficial if virtually all the gross tumor is able to be removed. Reliably determining in advance which patients are the best

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candidates has proven problematic. Current research efforts involve looking at ways to incorporate minimally invasive techniques to predict whether complete cytoreduction can be achieved, and thereby avoid the morbidity of laparotomy in patients likely to otherwise have a suboptimal debulking attempt. In these models, the primary elements suggesting unresectable disease, such as bulky carcinomatosis, mesenteric involvement, and extensive upper abdominal disease are evaluated prior to beginning cytoreductive efforts [10]. Vergote et al. reported one of the earliest attempts to utilize open laparoscopy in triaging patients to neoadjuvant chemotherapy (NACT) versus PDS [11].

Laparoscopic triage: In a pilot trial, Fagotti et al. described using sequential laparoscopy and laparotomy to predict surgical outcome, observing that laparoscopy could be employed with diagnostic accuracy [10]. In a follow-up study, these investigators recognized the need to set up a more objective quantitative model, and tested the statistical probability of various laparoscopic parameters to more accurately describe the burden of intra-abdominal disease. When present, each of seven features contributed two points to the predictive index value (PIV), and a PIV of ≥ 8 was inconsistent with being able to perform optimal cytoreduction (Table 1) [12]. The laparoscopy-based quantitative predictive index value established by Fagotti has since been validated and used in the Olympia-MITO 13 study, expanding the scoring system to satellite gynecologic oncology centers with 80% accuracy in all but one site [13, 14]. Institutional algorithms have also recently been published describing the use of diagnostic laparoscopy to assess the likelihood of optimal cytoreduction in advanced ovarian cancer [15].

Minimally invasive debulking: While the utility of minimally invasive techniques to triage patients most likely to benefit from a maximal cytoreductive effort is being studied, the question of whether staging, or even debulking of ovarian cancer can be done through modern laparoscopic techniques remains a hotly debated topic in gynecologic oncology. Unlike endometrial cancer where the Gynecologic Oncology Group (GOG) conducted the randomized LAP2 trial comparing laparoscopy to laparotomy for comprehensive staging of uterine cancer [16], no such comparable trial exists in the ovarian cancer literature. Instead, retrospective and institutional feasibility studies have evaluated the use of laparoscopy in early stage ovarian malignancy, finding no significant differences in spillage rates, upstaging, or survival when compared to traditional laparotomy [17-19]. The role of minimally invasive techniques in debulking primary or recurrent ovarian cancer is even more contentious, and until further investigation can be completed, the literature mainly supports minimally invasive surgery limited to the staging of early ovarian cancer and perhaps in assessing the feasibility of upfront cytoreduction in advanced cancer.

Upfront treatment of advanced disease

PDS followed by adjuvant chemotherapy has historically been considered the standard of care for all women with advanced epithelial

Omental cake
Peritoneal carcinomatosis
Diaphragmatic carcinomatosis
Mesenteric retraction
Bowel infiltration
Stomach infiltration
Superficial liver metastasis

Table 1: Fagotti laparoscopic predictive index value (PIV) score.

ovarian cancer. Many years of accumulated retrospective data suggest that achieving minimal residual disease leads to a better survival [8,20]. However, patients who have an unsuccessful or ‘suboptimal’ cytoreductive attempt do not necessarily benefit from the initial surgery in terms of overall survival [4]. Despite advances in surgical technique, morbidity is high and postoperative mortality also a concern, especially in the medically compromised population. NACT has proven to be a reasonable option for many solid tumors, such as breast cancer, rectal cancer, and head and neck malignancies [21,22]. Patients typically first undergo biopsies to confirm the diagnosis, and then the patient is treated with upfront chemotherapy. NACT has been an option for the treatment of ovarian cancer since the 1970s, but was infrequently used until about the last decade.

Recent trial results have challenged the dogmatic PDS approach, and the role of NACT continues to evolve. For some patients, NACT may not be followed by any surgical intervention due to non-response or medical infirmity, but the majority follow a general schema of three to four courses of primary chemotherapy, followed by interval debulking surgery (IDS), and then finishing with another three to four cycles of chemotherapy. The original data regarding NACT was largely from retrospective studies.

Retrospective data: In one such analysis, Jacob et al. compared 22 women (Group A) who underwent NACT followed by IDS plus chemotherapy to two control groups: Group B consisted of 22 patients who underwent suboptimal cytoreductive surgery and Group C were 18 patients referred after initial laparotomy and aborted PDS who were then immediately re-explored for an attempt at maximal cytoreduction. Both Groups B and C received six courses of adjuvant chemotherapy. Intriguingly, no significant difference were observed in median overall survival (OS) among the three groups (16 versus 19 versus 18 months, respectively; $P = 0.58$). However, 77% of Group A had minimal residual disease, compared to only 39% in Group C ($P = 0.02$). Furthermore, Group A patients who underwent optimal IDS appeared to have a longer median OS than those who did not (18.1 versus 7.5 months; $P = 0.02$) [23].

In another retrospective study using historical controls, Schwartz et al. reported the long term survival of 59 patients undergoing NACT. Forty-one patients subsequently underwent IDS. The study group received a median of five cycles of platinum-based NACT. The control group consisted of 206 consecutive women with stage III and IV epithelial ovarian cancer who were treated with PDS followed by a minimum of 6 cycles of platinum-based chemotherapy. Over 50% of the control group had a suboptimal surgical effort, defined as > 1 cm of residual disease, after PDS. There was no statistical difference in the OS or progression free survival (PFS) between the study and control group. Furthermore, the study group was significantly older (median age 67 versus 60 years; $P < 0.001$), and had a significantly worse performance status ($P < 0.001$). Interestingly, patients who underwent NACT and IDS had a longer overall survival compared to those who only received NACT (1.47 yrs versus 0.64 yr; $P < 0.0001$) [24].

Despite these promising findings, the impact of NACT remained controversial. To further explore the clinical ramifications, Bristow and Chi performed a meta-analysis of platinum-based NACT followed by IDS, analyzing 21 studies meeting their inclusion criteria. The mean weighted overall survival was 24.5 months. Of interest, the median survival increased 1.9 months with each 10% increase in the rate of maximal cytoreduction, and median survival was also increased with usage of taxane chemotherapy. However, with each incremental increase in the number of NACT cycles, the median survival was

decreased by 4.1 months. They concluded that NACT in lieu of PDS was associated with inferior overall survival [25].

Phase III trials: In hopes of settling the clinical debate, Vergote et al. conducted what they hoped would be a definitive phase III trial. As reported in 2010, 670 women with stage IIIC or IV epithelial ovarian cancer were randomized to either PDS or NACT. Those patients undergoing PDS then received a minimum of six cycles of platinum-based chemotherapy. NACT participants had three cycles of platinum-based treatment, followed by IDS and at least another three cycles. The study was powered to determine whether NACT was inferior to PDS with a primary endpoint of OS. In the event, the hazard ratio for death in the NACT group, compared to the PDS group was 0.98 (90% confidence interval (CI), 0.84 to 1.13, $P = 0.01$ for non-inferiority). Yet, residual tumor ≤ 1 cm was only achieved in 41.6% of patients undergoing PDS, but 80.6% who underwent NACT-IDS. Notably, of the PDS group, 2.5% died within a month after surgery, 7.4% had grade 3 or 4 hemorrhage from surgery, and 2.6% developed a thrombotic event. In comparison, the NACT group had reduced morbidity, with $\leq 1\%$ experiencing postoperative death, 4.1% having grade 3 or 4 hemorrhage, and no reported thrombotic events. Additionally, quality of life measures were not significantly different between groups. Based on these results, the investigators concluded that NACT followed by IDS was not inferior to PDS followed by chemotherapy [26].

The MRC CHORUS trial is a similar attempt to determine whether NACT therapy is comparable to PDS. In this prospective phase III study, 276 patients were randomized to PDS followed by platinum-based chemotherapy and 274 patients were randomized to receive NACT, then IDS. As reported, the median OS for PDS was 22.8 months compared to 24.5 months for NACT with a hazard ratio 0.87 actually favoring NACT. Likewise, the median PFS for PDS versus NACT was 10.2 versus 11.7 months with hazard ratio 0.91, again favoring NACT. Of interest, 35% of patients who underwent NACT were subsequently cytoreduced to no gross residual disease, whereas only 15% of PDS patients had similar operative success. While these intriguing findings have been presented, the manuscript only recently yet been published.

Japan Clinical Oncology Group protocol 0602 is another phase III trial comparing the morbidity of PDS versus NACT followed by IDS. In this study, patients were randomized to standard treatment, consisting of PDS followed by eight cycles of platinum-based chemotherapy (149 patients), or four cycles of platinum-based NACT followed by IDS and four additional courses (152 patients). Preliminary findings suggest that patients in the NACT arm had significantly less bowel/organ resection, blood loss, albumin transfusion and grade 3-4 adverse events after surgery.

The manuscript is not yet in print despite the literature supporting lower surgical morbidity and its non-inferiority, NACT is still not universally accepted as the primary treatment for the majority of advanced stage epithelial ovarian cancers. Patient selection is arguably the most controversial issue. For example, should all patients with stage IIIC/IV disease be offered NACT or is there radiographic or surgical (i.e. laparoscopy) criteria that can reliably predict that optimal cytoreduction at primary surgical intervention will not be achieved? Furthermore, the number of cycles of NACT prior to IDS is still not defined, and if not all, then which patients would benefit the most from a maximal cytoreductive attempt? From an investigational point of view, NACT allows for longitudinal tissue collection and the potential for a more robust exploration of translational research objectives. Thus, future clinical trials will increasingly be incorporating the NACT approach with targeted interventions, biopsies before and after therapy, with combined clinical and molecular endpoints.

Intraperitoneal therapy

The concept of giving chemotherapy directly in the abdomen, at a high dose, for a disease that is generally limited to the intraperitoneal (IP) cavity was recognized decades ago. Two collaborative group trials, each with a different treatment schedule, showed survival benefits compared to the standard, and otherwise comparable platinum-based intravenous (IV) regimens [27,28]. Yet, both studies were widely criticized, IP therapy found few advocates, and providers generally shied away from the logistical hassles and additional toxicity of this approach. The tipping point was publication of GOG protocol 172, a definitive phase III trial. In this study, 429 women with stage III ovarian cancer undergoing optimal PDS were randomized to IV paclitaxel 135 mg/m² and IV cisplatin 75 mg/m² or the experimental IP regimen (Table 2). Due to heightened toxicity, only 42% of the IP group completed six courses of the assigned therapy. Despite many patients having to switch to the IV regimen, the median duration of overall survival for the group randomized to IP remained superior (65.6 versus 49.7 months; $P = 0.03$). The enthusiasm was somewhat tempered by a worse quality of life during, and for several weeks after, IP chemotherapy [9]. Yet as a result of the dramatic difference in clinical outcome, a clinical announcement from the National Cancer Institute officially endorsed IP therapy in optimally debulked patients with stage III ovarian cancer.

For a number of reasons, including unsatisfying personal experiences from patients receiving IP therapy and healthy skepticism of the findings, many providers did not embrace this revived approach while others immediately set about revising the published drug schedule in all sorts of ways to make it a more attractive option for patients. Currently, we do not know which portion of the IP dosing results in the improved outcome. Thus, it is very difficult to know whether patients receiving a modified regimen will have reduced benefit, or not.

Ongoing phase III trials: The GOG conducted protocol 252 in hopes of settling some of these questions. Following optimal surgical debulking, more than 1500 patients were randomized to one of three regimens, all of which included bevacizumab during the 6 courses of therapy, then for about one year afterwards. The control arm consisted of a modified GOG 172 regimen commonly in use, with IV paclitaxel given over 3 hours (instead of 24) and IP cisplatin (at a reduced 75 mg/m² dose) on day 1, followed again by IP paclitaxel on day 8. The two experimental arms each consisted of dose-dense (weekly) IV paclitaxel 80 mg/m² and carboplatin (area under curve; AUC 6) every three weeks, given either IV or IP. Opened in 2009, the study quickly met enrollment, but results are still maturing.

The Intraperitoneal Therapy For Ovarian Cancer with Carboplatin (iPocc) trial is another randomized study of stage II-IV ovarian cancer after PDS that compares dose-dense paclitaxel and either IV or IP carboplatin. Thus far, more than three-quarters of the planned 605 patients have been enrolled. Of interest, fully two-thirds had a suboptimal debulking effort, with significant residual disease at randomization. Theoretically, IP therapy was only supposed to have beneficial impact for tiny implants of at most a few millimeters in size, through which it could infiltrate into the cells. However, in GOG 172, the IP regimen appeared to have a significant improvement regardless of tumor size. It is hoped that the iPocc study will settle the controversy of whether IP therapy works even in the presence of gross residual

Day 1:	intravenous paclitaxel 135 mg/m ² over 24 hours
Day 2:	intraperitoneal cisplatin 100 mg/m ²
Day 8:	intraperitoneal paclitaxel 60 mg/m ²

Table 2: Drug schedule for Gynecologic Oncology Group protocol 172.

disease. Further studies of IP bevacizumab, hyperthermic treatments, and other novel regimens will further clarify the best schedule [29,30].

One presumed disadvantage of IP therapy is that it only applies to patients who undergo successful PDS. While the iPoc trial may resolve the lingering question of its utility in suboptimally debulked patients, what about the burgeoning population treated by NACT? Is it practical, and does it work to put in an IP catheter at the time of IDS? A few phase II studies suggest that this is feasible and may lead to outcomes that are better than strict IV chemotherapy, but not as good as when given after PDS, as intended [31]. This topic is an attractive one for further study.

Maintenance therapy

A combination of aggressive surgery, before or after platinum-based chemotherapy, will induce a complete clinical remission in approximately 75% of patients with advanced ovarian cancer. Yet, even in this best responding group, up to 80 to 90% will relapse. Discovery of recurrence usually implies that the disease has become incurable. Could extended treatment improve this much dreaded result? Maintenance chemotherapy has been proposed as a strategy for providing additional treatment to patients who achieve a complete clinical response in order to sustain and prolong the time in remission. The primary goals of maintenance therapy therefore primarily include improvement in progression-free, symptom-free and OS intervals. Multiple drug schedules have been tested in ovarian cancers, including the use of cytotoxic and biologic agents, intraperitoneal chemotherapy, and even radiotherapy.

Cytotoxic drugs: Early maintenance chemotherapy trials employed the long-term usage of alkylating agents after completion of the initial chemotherapy regimen in an effort to prolong response [32]. Unfortunately, side effects were unacceptably burdensome, with patients experiencing significant concomitant toxicities of emesis, fatigue, and myelosuppression. Additionally, secondary malignancies were more likely to develop from prolonged treatment with these DNA-damaging agents [33]. Prolonged use of platinum agents for maintenance therapy (cisplatin or carboplatin) failed to show improvement in either PFS or OS in several trials, compared with the traditional treatment length of these regimens [34-36]. Similarly, toxicity of prolonged treatment, including emesis, renal toxicity and neurotoxicity, was greater with extended treatment.

Paclitaxel, due to its cell-cycle specific activity, anti-angiogenic properties, lack of induction of secondary malignancies, and potential patient tolerability of long-term treatment, became an attractive option as a maintenance drug, leading to its incorporation in several clinical trials. Certainly, it seemed more suitable than the alkylating and platinum agents. In a phase III trial, The SouthWest Oncology Group/GOG randomized women achieving complete remission to receive either three or 12 additional cycles of monthly paclitaxel [37]. The study was actually stopped early, while still accruing, after a planned interim efficacy analysis showed a significant improvement in PFS (28 versus 21 months; $P = 0.0035$) favoring the treatment arm receiving 12 cycles. Because only about half of the planned accrual was completed, and because patients who received 3 cycles of paclitaxel were allowed to cross over to 12 cycles of treatment, analysis of overall survival analysis was not feasible. However, no paradigm shift towards maintenance therapy resulted from this 'positive' study.

Furthermore, a subsequent Italian trial of maintenance paclitaxel, in a group of patients with similar characteristics, failed to show improvement in PFS or OS [38]. Despite incongruent clinical outcomes, observed neurotoxicity was substantial in both trials, with patients

experiencing more toxicity with the longer treatment regimens in each trial, requiring routine dose reductions. To determine if these side effects could be abrogated, the GOG has recently completed accrual on another large phase III maintenance paclitaxel clinical trial (protocol 212). In this study, patients who achieved a complete clinical response after primary therapy for stage III or IV disease were randomized to either no further therapy (observation arm), 12 cycles of reduced dose (135 mg/m^2) monthly paclitaxel, or a 12 cycle monthly dose of a novel microparticle-bound paclitaxel. The results of this trial should be available in a few years.

Biologic agents: Standard cytotoxic drugs demonstrate systemic, and often cumulative side effects, which makes biologic agents and other alternatives more attractive. Bevacizumab, a human monoclonal antibody to vascular endothelial growth factor (VEGF), is one such drug with a toxicity profile that appears conducive to utility as a maintenance therapy. In two reported phase III trials, bevacizumab was tested in combination with standard IV carboplatin and paclitaxel chemotherapy, then continued as a maintenance drug for a year afterwards. In GOG-218, all patients received 6 courses of IV carboplatin and paclitaxel, with either 1) placebo during cycles 2 through 6, and placebo for 16 cycles of triweekly maintenance; 2) bevacizumab (15 mg/kg) during cycles 2-6, followed by placebo, or 3) bevacizumab throughout. The median PFS was 10.3 months in the control group, 11.2 in the bevacizumab-initiation group, and 14.1 in the bevacizumab-throughout group [39]. The ICON7 trial compared six courses of standard IV carboplatin and paclitaxel to the same regimen with bevacizumab (7.5 mg/kg ; half the dose used in GOG-218), then every 3 weeks for an additional 12 cycles. The PFS at 42 months was 22.4 months without bevacizumab versus 24.1 months with bevacizumab ($P=0.04$) [40]. Importantly, no differences in OS were observed in either study. Additionally, an excess of clinically obvious relapses in the bevacizumab arm occurred immediately after discontinuation of maintenance therapy, suggesting that more extended use may be preferable. At a minimum, these trials demonstrated that bevacizumab is somewhat efficacious as a maintenance drug, but the dose and schedule require further study. Importantly, adverse events, including rates of hypertension requiring medical intervention, bleeding, and potentially catastrophic bowel perforation were more common in the bevacizumab treated groups in these trials.

Other options: Immunologic, vaccine, and targeted therapies such as interferon, tumor vaccines, and erlotinib have been also studied as maintenance therapies without demonstrable success. Numerous maintenance chemotherapy regimens have shown improvement in PFS, but to date, none has demonstrated improvement in OS. However, evaluation of long-term outcomes following maintenance therapy is limited by the spectrum of interventions utilized in the treatment of recurrent disease, drug crossover during trial participation, and use of the study maintenance drug in subsequent therapy of placebo-treated patients. Furthermore, selection of rational maintenance therapies must consider the impact of symptoms, quality of life, and cost. The ideal agent would be orally delivered, with minimal symptoms, relatively cheap, and demonstrating a clear clinical benefit. That is a tall order.

Rare tumors

With the emergence of molecularly based understanding of ovarian malignancies, it has become clear that reliance upon light microscopy of tumor tissue and resultant cell type-based classification is often overly simplistic. Kurman and colleagues have proposed a new system to lump epithelial ovarian cancer into two broad categories [42]. Type I malignancies include ovarian clear cell carcinoma (CCC), mucinous

and low-grade serous ovarian cancer. In general, these tumors often present confined to the ovary and share similar genetic alternations. These also seem to arise from a precursor lesion, often co-existing borderline tumors or endometriosis, by malignant transformation that includes a series of mutation events. Type II tumors include high-grade serous ovarian cancer (HGSOC), undifferentiated tumors, and carcinosarcomas. These malignancies tend to present at advanced stage with carcinomatosis and have different but more variable genetic alternations.

Large cooperative group trials establishing the current standards for ovarian cancer chemotherapy have traditionally been quite inclusive of different histologic subtypes. With high-grade serous and endometrioid carcinomas accounting for upwards of 80% of patients on trial, the reported outcomes do not necessarily reflect the less commonly occurring tumors, especially the rarest types. The benefit of any particular regimen is impossible to interpret, even in trials with thousands enrolled, amongst the rare tumors. For example, in the largest upfront treatment trial of epithelial ovarian cancer (GOG 182/ICON 5), CCCs and mucinous adenocarcinomas accounted for 3.3% and 1.5% of patients, respectively [43]. Consequently, without any other data to the contrary, treatment paradigms for the rare tumor histologies have heretofore been lumped with the more common variants.

With greater understanding of molecular events, it has become clear that a 'one size fits all' approach to epithelial ovarian cancer using platinum and taxane-based chemotherapy may not be the most effective treatment for all histologic subtypes. Moreover, as genomic data emerges in concert with new targeted therapies, opportunities have arisen for targeted therapy trials in this population. Given the low prevalence of these malignancies, a cooperative effort among gynecologic oncology centers in the United States as well as international partnership is particularly important in ensuring the feasibility of this research. The GOG Rare Tumor Working Group was established in 2005 with this in mind. The Gynecologic Cancer InterGroup (GCIg) has followed suit with its own initiative on rare gynecologic tumors.

With some exceptions, the upfront surgical management is largely the same, regardless of histology. However, in contrast to typical HGSOC, ovarian CCC, mucinous, and low-grade serous tumors are more commonly found confined to the ovary [44,45]. Yet, comprehensive staging is still essential to guide postoperative management.

Clear cell carcinoma: Ovarian CCC has a better prognosis, overall, than HGSOC as they are more often stage I at diagnosis and thereby carry a good prognosis, especially without capsular rupture. However, once the capsule is violated, stage for stage, prognosis is worse [46,47]. Currently, without a proven superior alternative, IV tri-weekly paclitaxel and carboplatin remains the standard of care. Even though ovarian CCC has been lumped with other histologic variants into studies of intraperitoneal and dose-dense regimens, it is hard to know whether the same clinical benefits apply. What is known is that CCC demonstrates more taxane/platinum chemoresistance, compared to high grade serous and endometrioid carcinomas.

Few chemotherapy trials have been limited to CCC, but since this histologic type is more common in Asian women, Japanese investigators have taken the lead on several of the CCC trials. The Japanese Gynecologic Oncology Group/GCIg collaborative study was a frontline randomized phase III trial of ovarian CCC comparing standard paclitaxel and carboplatin to cisplatin and irinotecan. Although the two-year PFS was similar ($P = 0.3$) based on unpublished

preliminary data, it does provide a basis for an alternate regimen that may be useful in selected circumstances.

The landmark profiling study of Zorn et al. demonstrated gene expression similarity of CCCs arising in the ovary, endometrium, and kidney [48]. For the most part, anti-angiogenic, mTOR pathway inhibitors, and other targeted therapies have replaced traditional cytotoxic chemotherapy in the treatment of renal cell carcinoma. Based on similarities in the molecular pattern, there is optimism that a parallel strategy could be implemented for ovarian CCC. In collaboration with Japanese investigators, the GOG has completed a phase II trial (protocol 268) as upfront therapy in this population. Ninety patients with stage III-IV ovarian CCC received standard paclitaxel and carboplatin with the mTOR inhibitor temsirolimus, followed by temsirolimus maintenance therapy. In the recurrent setting, the GOG has completed accrual of a phase II trial (protocol 254) of sunitinib. Data are still maturing for both studies, but they represent an exciting new approach to treatment of rare tumors. Currently, GOG 283 is underway, a phase II trial of dasatinib in recurrent or persistent CCC with loss of BAF250a.

Mucinous carcinoma: While benign mucinous cystadenomas and borderline tumors are fairly common ovarian neoplasms, frankly malignant adenocarcinomas are very rare. In fact, the vast majority resemble intestinal epithelium, and most of the tumors found in the ovary are actually metastases from the gastrointestinal tract or other organs [49]. When encountered, real ovarian mucinous carcinomas are usually confined to the ovary and, as a result, these stage I tumors carry an excellent prognosis. A single-institution retrospective review of 93 cases showed no instances of lymph node metastasis, consequently, routine lymphadenectomy can be omitted from the staging protocol [50]. However, more advanced mucinous tumors connote a worse prognosis compared to HGSOC, again due to chemo-resistance to standard carboplatin and paclitaxel.

As gene expression profiling has shown homology with adenocarcinomas of the lower gastrointestinal tract, treatment with agents traditionally used for these malignancies was postulated to be an attractive alternative. Accordingly, the GOG in cooperation with GCIg designed a definitive four-arm 'intergroup' multicenter phase III trial randomizing patients to carboplatin and paclitaxel versus oxaliplatin and capecitabine, each with or without bevacizumab for stage II-IV or recurrent mucinous ovarian cancer. Unfortunately, the study was closed prematurely due to poor accrual. Initially designed as a 350-plus patient trial, it only enrolled 50 and was not on pace to be completed. What data there is will be presented in due course.

Low grade serous ovarian cancer: Amongst the commonly encountered ovarian serous carcinomas, the low grade variant is relatively rare, and has unique features. As clinical findings have merged with evolving molecular data, it has become increasingly clear that subdivision of the low grade subtype as a separate entity is indicated [51-53]. These tumors appear to have a distinct oncogenic molecular pathway when compared to HGSOC [42]. For example, low-grade serous ovarian cancer is noteworthy for MAP kinase pathway alterations, with frequent BRAF and KRAS mutations. This is in contradistinction to HGSOCs, which characteristically have a hallmark p53 signature. Moreover, BRCA inactivation via mutation or promoter hypermethylation does not occur with regularity in low-grade tumors, but is commonly found in HGSOC. From an etiologic perspective, low-grade serous ovarian cancers are on the spectrum of serous borderline ovarian tumors, while many or most HGSOCs are thought to be derived from malignant transformation of the fimbriated end of the

fallopian tube. Clinically, low-grade patients tend to be younger, with prolonged survival compared to HGSOc.

Surgical management of primary disease and recurrences has traditionally been more important than systemic therapy. Fader and colleagues reviewed GOG 182 patients with grade 1 serous ovarian cancer and found that cytoreduction in advanced stage patients to no gross residual improved PFS and OS [54]. A recent retrospective study also showed significant improvement in PFS and OS in patients who underwent secondary debulking surgery that resulted in no gross residual disease [55].

Typically, low-grade tumors tend to be much more chemoresistant to the standard platinum-based chemotherapy used in upfront and recurrent HGSOc [56,57]. However, estrogen and progesterone receptors are commonly expressed and low-grade variants have been treated with endocrine agents anecdotally and in small series. More recently, one of the largest reports was published by Gershenson and colleagues of patients treated with varying regimens, including tamoxifen, aromatase inhibitors, progestins, gonadotropin-releasing hormone agonists, and combinations. The overall response rate was 9%, but with a promising 6 month PFS occurring in 61% of the regimens used [58].

In one of the first prospective clinical trials exclusively enrolling low-grade serous ovarian cancers, the GOG conducted a phase II study of selumetinib (a MEK inhibitor) in patients with recurrent disease (protocol 239). Of the 52 patients accrued, the response rate was 15.4%. However, 63% experienced at least 6 month PFS. Overall, toxicity was acceptable, but 25% of patients ultimately came off study due to toxicity [59]. Regardless, these data compare favorably to the efficacy and toxicity of standard chemotherapy in this disease. Tumors with the V600E BRAF mutation have been found to be more likely to behave in a benign fashion [60]. In GOG 239, where all patients were recurrent and 57% of patients had three or more prior regimens, only 6% of evaluable patients had BRAF mutations [59].

PARPi/BRCA testing: Germline mutations in BRCA1 and BRCA2 genes confer an increased risk of ovarian cancer, particularly the most common type: HGSOc. BRCA1 and BRCA2 encode tumor suppressors involved in repairing double-stranded DNA breaks via homologous recombination (HR) and maintaining genomic stability [61]. About 15% of epithelial ovarian cancers are deficient in HR, due to mutations in BRCA1/2. In up to 50% of patients with HGSOc, the tumor cells are deficient in HR due to germline or somatically-acquired BRCA1/2 mutations, epigenetic silencing of BRCA, or defects in other genes such as RAD51, ATM, ATR, CHK1/2, and Fanconi anemia pathway genes [62].

Poly (adenosine diphosphate [ADP]-ribose) polymerase (PARP) plays an essential part in the repair of single-stranded DNA breaks, through the base-excision repair pathway, and it has been proposed that PARP keeps low-fidelity nonhomologous-end-joining DNA repair machinery in check. PARP inhibition leads to the formation of double-stranded DNA breaks that cannot be accurately repaired in tumors with HR deficiency, causing synthetic lethality in the tumor cells [63].

Multiple PARP inhibitors, including olaparib, veliparib, niraparib, rucaparib and BMN673, are currently in testing for treatment of ovarian cancer [64]. All are administered orally. Olaparib has undergone the most extensive clinical investigation and was the first to be tested in a phase I trial where the maximum tolerated dose was found to be 400 mg, twice daily (BID), in capsule form [65]. Lederman et al. conducted a randomized, placebo-controlled, phase II trial

(Study 19) evaluating olaparib maintenance in patients with relapsed platinum-sensitive HGSOcs. Of 265 patients, 136 were randomized to olaparib 400 mg capsules BID. PFS was notably improved in the experimental group (8.4 versus 4.8 months; HR 0.35; $P < 0.00001$) [41]. A preplanned retrospective analysis showed that olaparib provided the greatest benefit to patients with a BRCA mutation. Additionally, the increase in PFS by olaparib was more pronounced in patients with either a germline or tumor BRCA mutation (11.2 versus 4.3 months; HR 0.18; $P < 0.0001$) than in patients without a BRCA mutation (7.4 versus 5.5 months; HR 0.54; $p=0.0075$) [66]. In studies that evaluated the efficacy of olaparib monotherapy, the objective response rate (ORR) ranged from 23% to 69%, and the higher ORR was correlated with platinum sensitivity [67-69]. Kaye et al. found that the ORR of olaparib was similar to pegylated liposomal doxorubicin [70]. These positive phase II studies have led to two phase III randomized controlled trials evaluating olaparib maintenance (300 mg tablets BID) in BRCA mutation carriers with HGSOc or endometrioid ovarian cancers, following first line platinum-based chemotherapy (GOG3004/SOLO1) or in relapsed platinum-sensitive patients following two previous platinum-based regimens (GOG3004/SOLO2). Both studies have recently reached accrual goal (344 and 264 patients, respectively). Two other PARP inhibitors, niraparib and rucaparib, are being studied in a similar fashion in the recurrent setting [64].

Olaparib appears to be well tolerated, with the most common side effects being nausea, vomiting, fatigue and anemia [41]. In December, 2014, the United States Food and Drug Administration granted accelerated approval of olaparib as monotherapy for the treatment of patients with deleterious or suspected deleterious germline BRCA mutations who have relapsed ovarian carcinoma after three or more prior lines of chemotherapy.

PARP inhibitors in combination with cytotoxic or targeted therapies are also being tested. Some examples of the targeted therapies include anti-angiogenic agents (bevacizumab, cediranib) and PI3K inhibitors (BKM120) [64]. It should be noted that with combined therapy, achievement of full dose chemotherapy is difficult because of the overlapping myelosuppressive toxicities. In a randomized phase II study, olaparib (200 mg BID x 10 days) concurrent with paclitaxel/carboplatin, followed by olaparib maintenance showed improved PFS (HR = 0.51, $p=0.0012$), with similar ORR compared to standard IV carboplatin/paclitaxel [71].

PARP inhibitors provide an exciting new therapeutic class for BRCA mutated ovarian cancers. Future research is needed to develop strategies to identify somatic BRCA mutations/HR-deficiency in ovarian tumors, to overcome PARP inhibitor resistance, and to optimize the sequence of how these agents are incorporated in clinical management (first line or relapsed, concurrent or maintenance). Yet increasingly, at many centers, all patients with epithelial ovarian cancer are being encouraged to undergo genetic testing to determine if they might be a future candidate for PARP therapy in the presence of a germline BRCA mutation.

Ovarian cancer stem cell inhibition

The cancer stem cell (CSC) hypothesis postulates that tumors contain a quiescent population of cells that are capable of both self-renewal and generation of differentiated progeny with limited proliferative capacity [72]. Many investigators have exploited differential cell surface marker expression or differences in biochemical properties to isolate and characterize CSCs from human ovarian tumors [73-75]. In pre-clinical studies of ovarian cancer, these cells have been shown to persist following treatment with conventional chemotherapeutics that target

rapidly dividing cells, suggesting that the inherent chemoresistance of CSCs enables them to withstand standard cytotoxic chemotherapy and drive disease recurrence [76,77]. Significantly, expression of the ovarian CSC markers CD133 and ALDH1 has been linked to poor prognosis [78,79]. Finding effective therapies that target the critical ovarian CSC cell population remains a challenge though promising new avenues of investigation have recently been described [80,81].

Several pre-clinical studies have analyzed the efficacy of direct targeting of ovarian CSCs. Tumor growth in an ovarian cancer mouse model was limited by IP treatment with an anti-CD133 antibody conjugated to pseudomonas exotoxin 38, suggesting that therapeutic targeting of the CD133+ stem cell population is sufficient to restrict disease progression [82]. The ovarian CSC marker ALDH1 also functions as a mediator of the stem cell phenotype and as such is a viable target for therapy. Landen et al showed that elimination of ALDH1A-1 expression via siRNA silencing re-sensitized chemoresistant ovarian cell lines to platinum and paclitaxel, and resulted in reduced tumor growth in an *in vivo* orthotopic mouse model (7). Similar epigenetic strategies have yielded promising results in the clinic.

In a phase II study, the hypomethylating agent decitabine altered global and gene-specific DNA methylation, leading to a high response rate and prolonged PFS in patients with recurrent platinum-resistant ovarian cancer [83]. More recent data studying the effect of the hypomethylating drug SGI-110 in pre-clinical models of ovarian cancer suggest that it specifically targets the ALDH1+ CSC population with marked effects on the cells' ability to initiate tumor formation, remain resistant to chemotherapeutics and maintain an undifferentiated state. Administration of SGI-110 after completion of carboplatin treatment inhibited growth of ALDH+ ovarian CSCs and decreased tumor progression, suggesting that clinical strategies involving epigenetic targeting of CSCs may help to prevent disease recurrence following completion of standard platinum based chemotherapy [84].

Several therapeutic strategies in many tumor types have focused on impeding CSC function by inhibiting transduction pathways [85]. These signaling cascades are known to be aberrantly regulated in ovarian cancer and contribute to the chemoresistance of ovarian CSCs [86-89]. A few trials in ovarian cancer have focused on investigating the efficacy of agents targeting specific pathways of carcinogenesis.

Notch pathway: The Notch pathway has been shown to be activated in 22% of HGSOs, making its dysregulation a potential means of stratifying patients for targeted therapy [62]. McAuliffe et al determined that inhibition of Notch signaling sensitizes ovarian cancer cell lines and mouse models to platinum therapy by targeting CSCs [90]. Consistent with these results, inhibition of Notch signaling in patient derived xenografts generated from women with platinum-resistant disease demonstrated that the addition of a gamma secretase inhibitor enhanced the response of these tumors to single agent paclitaxel [91]. Clinical trials testing the efficacy of Notch pathway inhibition against tumors across many disease sites have revealed the complexity of targeting this pathway [92]. One early trial assessing the Notch inhibitor MK7052 in solid tumors including ovarian, fallopian tube and peritoneal carcinomas was terminated before completion, most likely due to significant toxicities associated with the inhibitor. Notch pathway inhibition, however, remains a promising strategy to target the ovarian CSC and future studies will require the development of safe and effective therapeutics.

Hedgehog pathway: In CSCs, Hedgehog signaling has been shown to contribute to self-renewal, tumor growth and metastasis and chemoresistance, suggesting Hedgehog inhibitors may be clinically

useful [93]. Several investigators have looked at the effect of Hedgehog pathway inhibition in pre-clinical *in vivo* studies of ovarian cancer. Analysis of the Hedgehog pathway inhibitor IPI-926 on the growth of serous ovarian patient-derived xenografts demonstrated efficacy as a consolidation therapy that blocked resurgence of tumor growth following cessation of paclitaxel and carboplatin treatment, although a specific effect on ovarian CSCs was not determined [94]. Later studies examining the consequence of Hedgehog pathway inhibition on the growth of xenografts derived from paclitaxel-resistant ovarian cancer cell lines determined that the combination of paclitaxel and the Hedgehog inhibitor LDE225 reduced tumor burden more effectively than treatment with either drug alone [95]. These data suggest that Hedgehog inhibition may help to resensitize cells to chemotherapy and be a successful treatment strategy in ovarian cancer. In a phase IB/II trial, the efficacy of Hedgehog inhibition via LDE225 and paclitaxel in women with platinum resistant ovarian cancer who have received prior platinum and taxane based therapies is under investigation with an estimated completion date in 2017.

Jak/STAT pathway: Finally, the Janus kinase/signal transducer and activator of transcription (Jak/STAT) pathway is well established as a critical mediator of tumorigenesis due to its constitutive activation in many human malignancies [96]. In ovarian cancer, this pathway is aberrantly activated with specific enhanced signaling in chemoresistant cells and is associated with disease progression and poor prognosis [97-99]. A number of therapeutics directed against the Jak/STAT pathway have been developed with many currently being evaluated across multiple disease sites [96]. For example, the oral Jak kinase inhibitor ruxolitinib, which has been approved for treatment of myelofibrosis, is being tested in patients with advanced lung, breast colorectal and pancreatic malignancies [100]. Recent studies have examined the effect of Jak inhibitors on the progression of ovarian cancer in a pre-clinical mouse model of peritoneal dissemination. This analysis determined that blockade of Jak kinase activity leads to reduced peritoneal tumor growth and decreased ascites production [101]. Jak inhibition in a transgenic mouse model of ovarian tumor development and progression shows similar diminishment of tumor volume and ascites production [102]. Taken together, these pre-clinical studies suggest that inhibition of Jak/STAT pathway activity may be an effective strategy in the clinical management of recurrent ovarian cancer. Although the Jak/STAT signaling pathway is critical to CSC function in breast (44, 45) and prostate tumors (46), it remains to be determined if inhibition of Jak signaling specifically targets the ovarian CSC [103-105].

Immunotherapy (PD-1)

The treatment of cancer is evolving to include immunotherapy designed to harness a patient's own defenses against tumor cells. Proven strategies in the treatment of ovarian cancer have included the passive transfer of monoclonal antibodies targeting cancer-associated proteins (such as VEGF and EGFR), yet a body of literature is growing which suggests it is possible to modulate the body's endogenous immune response. Novel therapeutics have been designed to enhance the anti-tumor immune response or reverse immune suppression. One of the more promising strategies is the manipulation of cell surface molecules, known as immune checkpoints, which when activated can either disrupt or augment a particular immune response to a tumor antigen.

T-cell activation is regulated through a balance of positive and negative signals provided by such co-stimulatory checkpoint receptors. It has been well established that tumors resist immune attack by inducing tolerance among tumor-specific T cells via the expression of ligands that bind to inhibitory receptors [106]. One of the most

important checkpoint pathways is the programmed death-1 (PD-1) pathway. Activation of the PD-1/PD-L1 axis causes a down-modulation of T-cell activation resulting in a decrease in antitumor immunity. The PD-1 protein is a receptor that is expressed on T cells and binds to its ligand PD-L1/L2 that is selectively expressed on many cancer cell lines – including ovarian [107]. PD-L1 expression has furthermore been found to correlate with poor prognosis [108,109]. Blocking the binding of this ligand to its receptor is thus theoretically capable of reversing T-cell suppression and ultimately inducing an anti-tumor response.

Nivolumab, a fully human monoclonal antibody against PD-1, was the first PD-1 inhibitor to be approved worldwide. Consistent with the proposed mechanism of action, a phase I study of patients with locally advanced solid malignancies treated with nivolumab found ORRs across dose cohorts, as measured by standard RECIST criteria, ranging from 6% to 32% in non-small cell lung cancer, 19% to 41% in metastatic melanoma and 24% to 31% in renal-cell cancer. Most of the responses were durable and the toxicity profile was found to be safer than ipilimumab (a monoclonal antibody targeting CTLA4, another inhibitory molecule expressed on activated T-cells recently approved by the FDA for advanced melanoma patients) [110]. While data continues to accumulate in favor of the use of nivolumab in the treatment of cancers such as melanoma or renal cell carcinoma, limited data exist to guide the use of nivolumab in the treatment of ovarian cancer.

Hamanishi et al. recently presented unpublished preliminary data from a phase II efficacy trial. Twenty women with platinum-resistant recurrent or refractory ovarian cancer were administered nivolumab every two weeks at doses of 1 or 3 mg/kg. Patients received nivolumab for up to 6 cycles (4 doses/cycle) of treatment or until disease progression. At the time of the data cutoff, the clinical response rate was 17% with a disease control rate of 44%. There were only two grade three adverse events reported. Thus, the available ovarian cancer data, considered alongside favorable data from the treatment of other solid cancers, suggests that nivolumab is well tolerated and may have encouraging clinical efficacy for patients with advanced or relapsed, platinum resistant ovarian cancer.

It is likely that checkpoint inhibitors such a nivolumab will play an important role in the future of immunotherapy and in the treatment of ovarian cancer. The use of checkpoint inhibitors for women with advanced or metastatic ovarian cancer has the potential to increase the likelihood of a sustained, durable response while decreasing the toxicity seen with standard cytotoxic chemotherapy. Further clinical trials are needed to validate such preliminary findings, to identify which patients are likely to respond, to determine the appropriate dose of therapy, and to establish when in the course of treatment such medications should be employed.

Conclusions

Ovarian cancer is a molecularly heterogenous disease and the standard of care consisting of primary debulking surgery followed by adjuvant platinum-based chemotherapy should not be applied to every patient if we are to make real progress in the future treatment of this disease. Innovative therapeutics, delivered as targeted agents, are just beginning to come on-line in a series of early phase I/II trials that appear to be relegating costly, large phase III trials with thousands of enrolled patients receiving old-school cytotoxic drugs to historical interest. In order to meaningfully improve our patient's clinical outcomes and quality of life in a cost-effective manner, novel approaches to trial development, treatment paradigms and non-traditional drugs, such as immunotherapy, need to be implemented.

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