Personalizing therapy for ovarian cancer

The treatment of ovarian cancer is uniform for almost all patients, with advanced stage (IC–IV) generally treated with surgical debulking followed by adjuvant chemotherapy consisting of carboplatin alone or in combination with paclitaxel. However, current trends in cancer treatment have demonstrated the benefit of personalized therapies, which take advantage of differences in tumor cell types and genetics. The advantage of the personalization of treatment has already been demonstrated in other cancer types through the identification of patient subpopulations and the creation of genetic tumor profiles. It is increasingly likely that such an approach could prove helpful for ovarian cancer patients. Research regarding the heterogeneity of ovarian tumors with respect to both grade and histology, including the unique classification of clear-cell and low-grade tumors, has helped to further the concept that different tumors require individualized attention. Furthermore, advances in the understanding of the genetic basis for ovarian cancer have provided new insight into cancer-related mutations, including those found in the *BRAF*, *KRAS*, *PIK3CA* and *BRCA* genes, and provide targets for tumor-specific treatment regimens. The aim of this therapeutic approach is to minimize toxicities and optimize the chance of response and survival.

KEYWORDS: heterogeneity ■ microarray ■ ovarian cancer ■ personalized medicine ■ targeted therapy

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Ovarian cancer is the fifth largest cause of cancer-related death in females in the USA [1]. It is estimated that 15,000 women died from ovarian cancer in 2009. These dismal statistics result, in part, from the advanced stage at which ovarian cancer is diagnosed. The symptoms for ovarian cancer, which include abdominal or pelvic discomfort, urinary frequency, bloating or early satiety, are often nonspecific or reflect late-stage disease. Unfortunately, there is no effective early-detection test for the disease. A majority of ovarian cancer patients present with disease that has spread throughout the abdomen and lymph nodes. The overall patient 5-year survival rate is 46% and, unfortunately, this overall survival rate has only had minimal improvements in the last 20 years [2].

The standard initial treatment for ovarian cancer includes optimal surgical debulking followed by six cycles of adjuvant carboplatin and paclitaxel treatment [3]. For the majority of patients, the tumors will recur and they eventually develop resistance to chemotherapy. The emergence of many novel biologic agents targeting specific pathways in the tumorigenesis of cells, along with recent findings demonstrating the involvement of distinct molecular events in the development of the different histology subtypes and grades, warrant a closer look at the standard, upfront treatment regimen currently available to ovarian cancer patients. The standard 'one-size-fits-all' approach may underserve many ovarian cancer patients and more targeted therapy may improve overall patient survival rate.

Tumor heterogeneity mediates the value of individualized therapies

■ Non-small-cell lung cancer subpopulations

The potential benefits of personalized therapy for cancer patients have been demonstrated in the treatment of several other diseases, including breast and non-small-cell lung cancers (NSCLCs). Lung cancer accounts for 15% of all cancer cases in the USA, with 219,440 new cases in 2009, 85% of which can be classified as NSCLC. Lung cancer is also the deadliest cancer in the USA, representing 28% of all cancer-related deaths [2]. The standard of treatment for advanced-stage NSCLC has been combination platinum-based cytotoxic chemotherapy. Two commonly used therapies within this category are gemcitabine with cisplatin, and carboplatin with paclitaxel [4,5]. Both regimens demonstrate moderate response rates and increased time-to-progression compared with the best supportive care alone.

One relatively new approach towards treating NSCLC has been through the use of tyrosine kinase inhibitors (TKIs) of the EGF

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receptor (EGFR), which controls various intracellular signaling pathways related to cell proliferation and survival. Faulty EGFR signaling pathways have been implicated in numerous cancers, including lung cancer, and have resulted in the development of several EGFR–TKIs as possible treatments for these malignancies [6].

Gefitinib is a TKI that showed promising monotherapy response rates of 10–19%, as well as manageable toxicities, in Phase II trials [7,8]. Based on these positive results, two Phase III studies were performed to evaluate whether gefitinib in combination with traditional chemotherapy showed any increased antitumor activity over standard chemotherapy alone in patients with advanced NSCLC. Two studies, International Trial on Antiatherosclerotic Coronary Therapy (INTACT) 1 and 2, tested gefitinib with gemcitabine and cisplatin, and gefitinib with paclitaxel and carboplatin, respectively. Neither study showed any significant increase over chemotherapy alone with regard to overall survival, time to progression or tumor response [9,10]. Another EGFR–TKI, erlotinib, was also tested in two Phase III trials in combination with standard chemotherapy. The first, Tarceva Responses in Conjunction with Paclitaxel and Carboplatin (TRIBUTE), investigated erlotinib with paclitaxel and carboplatin, while Tarceva Lung Cancer Investigation (TALENT) studied erlotinib with gemcitabine and cisplatin. The results of both were consistent with the earlier INTACT trials: no significant increase in overall survival or time to progression over chemotherapy alone. However, unlike INTACT, the erlotinib studies were able to recognize a specific subpopulation of patients who demonstrated a significantly higher response to the erlotinib arm of the trial as opposed to the placebo. These were patients with NSCLC who had no significant history of smoking, termed 'never-smokers' [11,12].

Clearly, something about this subset of patients made their tumors more responsive to EGFR–TKIs. It had been hypothesized that higher levels of EGFR expression in some individual tumors may afford greater sensitivity to EGFR–TKIs. However, several studies have demonstrated that this was not the case. For example, Parra *et al.* concluded that a tumor's EGFR expression was not a good predictor of response to monoclonal therapy with gefitinib for patients with NSCLC [13]. Another hypothesis was that a specific EGFR gene mutation was occurring in never-smokers with NSCLC and increased their sensitivity to inhibitors. To test this, Pao *et al.* analyzed tumor cells from NSCLC patients to determine whether they exhibited any EGFR mutations. The study included three groups:

- Group 1, patients treated with gefitinib (n = 18);
- Group 2, patients treated with erlotinib (n = 17);
- Group 3, patients not treated with any EGFR–TKI (n = 96).

Groups 1 and 2 were further divided into drug-sensitive and drug-refractory (or insensitive) cohorts, while group 3 was divided into never-smokers (<100 cigarettes during lifetime) and current or former smokers. The researchers discovered that seven out of ten (70%) gefitinib-sensitive and five out of seven (71%) erlotinib-sensitive tumors had EGFR mutations in the tyrosine kinase (TK) domain, while none of the refractory tumors for either inhibitor contained mutations. Furthermore, seven out of 15 (47%) untreated never-smokers contained mutations in the TK domain, while only four out of 81 (5%) untreated smokers exhibited a mutation. Finally, while the mutations discovered so far do vary somewhat from patient to patient, 88% occur within two 'hotspots': multinucleotide in-frame deletions in exon 19 and point mutations within exon 21, both found in the EGFR–TK domain. Based on these results, Pao *et al.* concluded that there exists a specific genotype found in never-smokers that confers increased sensitivity to EGFR–TKIs [14].

Lynch *et al.* have discovered that in the presence of signal ligands, including EGF, mutated EGFR exhibits phosphorylation activation levels two- or three-times that of wild-type EGFR. These mutated receptors also remain activated for longer, usually several hours, compared with approximately 15 min for wild-type receptors [15]. However, more research is required, especially among different ethnic populations, to determine whether the results are universal. What is clear, however, is that clinical experiences with EGFR–TKIs provide an important example of how understanding the genetics of individual patients can lead to more precise and personalized therapy. By discovering the existence of patient subpopulations, such as NSCLC patients with EGFR mutations, more effective treatments can be selected, rather than using the same therapies for everyone.

Breast cancer gene profiling

In addition to lung cancer, the benefits of personalized therapy have also been realized in the treatment of breast cancer. The standard of care for early-stage, localized breast cancer has been surgery, with possible radiation, followed by systemic adjuvant therapy. Such adjuvant chemotherapies are intended to eliminate microscopic malignancies and have been shown to drastically increase a patient's chance of long-term survival. However, despite their benefits such treatments are associated with considerable toxicities, and it would be extremely helpful if patient subpopulations that do not require adjuvant therapy could be identified, thus sparing them the unnecessary side effects. Unfortunately, little information is available to help physicians decide which patients do not require additional chemotherapy, and as a result, the majority of breast cancer patients are treated similarly [16].

In 2002, van't Veer *et al.* tried to determine whether there was a specific gene profile that could help predict the likelihood of metastatic disease in breast cancer patients. Such a profile could then help physicians differentiate between patients who would probably benefit from adjuvant therapy and those who would not. For the profile, the researchers retrospectively selected tumors from 78 lymph node-negative patients: 44 had remained metastatic-disease free for at least 5 years following initial diagnosis (good prognosis); the remaining 34 had developed metastatic disease within the 5-year interval (poor prognosis). Using a microarray hybridization technique, 231 genes were discovered to be significantly expressed in the tumors; 70 of these genes were ultimately chosen for the profile. The tumors were then ordered and plotted against each other by comparing each individual gene profile to the average of all the good-prognosis tumors. Finally, a threshold line was set on the plot that would allow no more than a 10% misclassification of prognosis. Any tumor with a gene profile falling above that line on the plot was good prognosis and would not require adjuvant therapy. Tumors below that line were considered poor prognosis and would receive additional chemotherapy. The prognosis classifier was then tested on a separate cohort of lymph node-negative tumors (n = 19), resulting in only two of 19 patients (10.5%) being misclassified [17].

Since its creation, the 70-gene profile has been validated by several other studies. Buyse *et al.* looked at a cohort of 307 patients, aged 61 years or younger, with lymph node-negative breast cancer, and determined an unadjusted hazard ratio of 2.32 for time to distant metastases [18]. Mook *et al.* also determined that the prognosis signature was an effective tool in predicting metastasis in postmenopausal patients between the ages of 55 and 70 years [19]. Finally, van de Vijver *et al.* calculated a hazard ratio of 5.1 for distant metastases in the poor-prognosis group as compared with the good-prognosis group, in a cohort of 295 patients. The study also compared the 70-gene prognosis profile to the St Gallen criteria and NIH consensus criteria for the administration of adjuvant therapy in breast cancer patients. The researchers found that the 70-gene prognosis profile significantly outperformed the other two criteria by correctly identifying high-risk patients and correctly assigning many more patients to the good-prognosis category. Thus, patients who actually had a high probability of metastatic disease within 5 years were correctly identified, and patients who were incorrectly classified by St Gallen and NIH as high risk were appropriately categorized as not requiring adjuvant therapy [20]. Evidently, this gene profile is a powerful tool that can help physicians decide which patients need adjuvant therapy and which patients can go without, allowing for better patient-tailored treatments.

Heterogeneity in ovarian cancer

Ovarian cancer histology

Epithelial ovarian cancer is comprised of tumors with different histologies, including serous, mucinous, endometrioid and clear-cell tumors [21]. These subtypes have substantial clinical differences, including clinical presentation and overall patient survival [21]. A total of 75% of papillary serous carcinomas of the ovary are diagnosed in the advanced stages, while only 40% of mucinous, endometrioid and clear-cell carcinomas are diagnosed in the advanced stages (**Figure 1**) [22]. Among early-stage ovarian carcinomas, patients with endometrioid and mucinous tumors have a 10-year disease-specific survival of 85 and 79%, respectively, while those patients with clear-cell and high-grade serous tumors have a 10-year disease-specific survival of 70 and 57%, respectively [23]. However, compared with endometrioid and serous, clear-cell and mucinous tumors have a dramatically poorer prognosis in late-stage disease [1]. Despite this clear clinical and pathologic heterogeneity, all patients with these tumors are treated with surgery and standard chemotherapy [24].

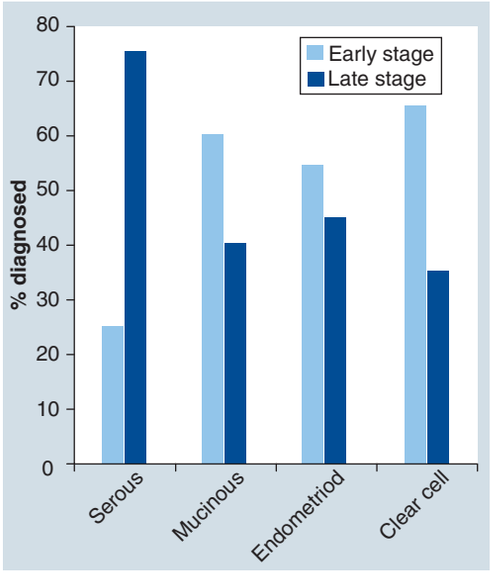

Figure 1. Stages of ovarian cancer cases at diagnosis by histology. The majority of papillary serous ovarian cancer cases are diagnosed in the late stages while the converse is true among mucinous, endometrioid and clear-cell carcinomas.

Recent advances in the elucidation of molecular signatures specific to ovarian histologic subtypes have provided a molecular basis on their varying clinical behavior. Using cDNA microarrays, Zorn *et al.* compared patterns of gene expression in 75 ovarian and endometrial tumors of endometrioid, serous and clear-cell subtypes (Figure 2) [25]. The gene-expression signatures of serous and endometrioid subtypes can be used to identify their organ of origin. However, this was not true for the clear-cell histotype, where there was substantial overlap in gene-expression profiles among endometrial clear-cell and ovarian clear-cell tumors, and this extended to renal clear-cell tumors. The study demonstrated that while the endometrioid and serous subtypes' gene-expression profiles were distinct with respect to their organ of origin, the clear-cell tumors exhibited similarity in gene-expression pattern regardless of the primary site [25]. This strongly suggested that ovarian clear-cell cancers are distinct from other ovarian cancers and should be treated separately.

The genomic characterization demonstrating the similarity in gene expression of ovarian, endometrial and renal clear-cell carcinomas supports the idea of targeting distinct molecular pathways in tumorigenesis rather than treatment solely based on the organ of origin. Targeting VEGF, a key player in tumor angiogenesis in renal clear-cell cancer [26], has demonstrated relative success and is a viable option for ovarian clear-cell carcinomas. Such active agents include: bevacizumab, a monoclonal antibody that binds to VEGF-A and, thereby, blocks ligand–receptor interaction; sunitinib, a receptor tyrosine kinase inhibitor of the VEGF pathway; and sorafenib, a multikinase inhibitor [27–30]. In the placebo-controlled study by Yang *et al.*, 10% of renal clear-cell patients achieved a 50% reduction in tumor size at the biweekly dose of 10 mg/kg of bevacizumab [27]. The progression-free survival of that treatment group was significantly longer ($p < 0.001$) than that of the placebo group, with a median time to progression of 4.8 versus 2.5 months. Motzer *et al.* demonstrated sunitinib's strong activity in patients with cytokine-refractory clear-cell renal cell carcinoma, with 36 out of 106 partial responses (34%; 95% CI: 25–44%) and 30 out of 106 (29%) stable disease for longer than 3 months [28]. The median progression-free interval was 8.3 months (95% CI: 7.8–14.5 months). Patients were treated on a 4 weeks on, 2 weeks off, 50 mg regimen. Finally, in the study by Staehler *et al.*, all five patients who achieved complete response from treatment with sorafenib or sunitinib with or without subsequent surgery had clear-cell histology and the median duration of complete response was at least 24 months [29].

Given the poor response rate of ovarian clear-cell cancer to platinum-based therapy [21], histology-specific approaches are needed. Studies designed with responses stratified by histology may provide better insight into the antiangiogenic-targeted approach for treating different ovarian cancer subtypes, particularly that of clear-cell histology. The first reported use of sunitinib in ovarian clear-cell adenocarcinoma was in a 60-year-old patient, but this was as a fifth-line therapy [31]. The patient did experience a decrease of cancer antigen 125 after 1 month of daily oral sunitinib at 50 mg, but quickly succumbed to the disease. It will be interesting to see results from the current clinical trials for ovarian clear-cell carcinoma, such as Gynecologic Oncology Group (GOG) 254 and GOG RTM 0907.

Ovarian cancer grade

Invasive epithelial ovarian cancers span a pathologic spectrum from low grade to high grade (1–3). In addition, borderline or low malignant potential (LMP) tumors (grade 0) of the ovary possess morphologic features of malignancy, including nuclear atypia and the ability to metastasize, but are not aggressive [32]. Despite these properties, patients with LMP tumors have an excellent 5-year survival even when

presenting with advanced-stage disease. Even with these differences in clinical behavior, all patients with invasive ovarian cancers are treated in a similar fashion to high-grade tumors.

Recent genomic analysis of LMP and invasive low-grade tumors has revealed that they are distinct from high-grade tumors. An analysis of gene expression of 66 samples from late-stage, high-grade serous ovarian carcinomas and LMP serous tumors revealed that both tumor types cluster into two distinct arms [33]. Furthermore, LMP tumors cluster more closely to normal ovarian serous epithelium than to late-stage, high-grade serous ovarian carcinomas. In addition, low-grade invasive tumors are essentially indistinguishable from LMP tumors (FIGURE 3). These data provided the genomic characterization that low-grade tumors and LMPs represent a separate disease from high-grade tumors. Upon analyzing differentially expressed genes, the study also reported coordinated dysregulation of genes that activate the wild-type p53 pathway. Elevated levels of the tumor suppressor *TP53,* its downstream effector *CDKN1A,* activators of p53, such as *PPM1A,* and decreased levels of inhibitors of p53, *UBE2D1* and *ADNP* were found in LMP tumors, but not in high-grade cancers [33]. Unlike LMP tumors, late-stage high-grade tumors expressed high levels of genes involved in cell proliferation and metastasis, such as *PDCD4, E2F3, MCM4, CDC20* and *PCNA* [33]. LMPs and low-grade serous tumors exhibit low expression of proliferation markers, such as *CDC2, KIF11, TOP2A, CCNB1* and *MKI67* [34]. The activation of wild-type p53 may explain, in part, the excellent survival of patients with these tumors compared with those with high-grade cancers.

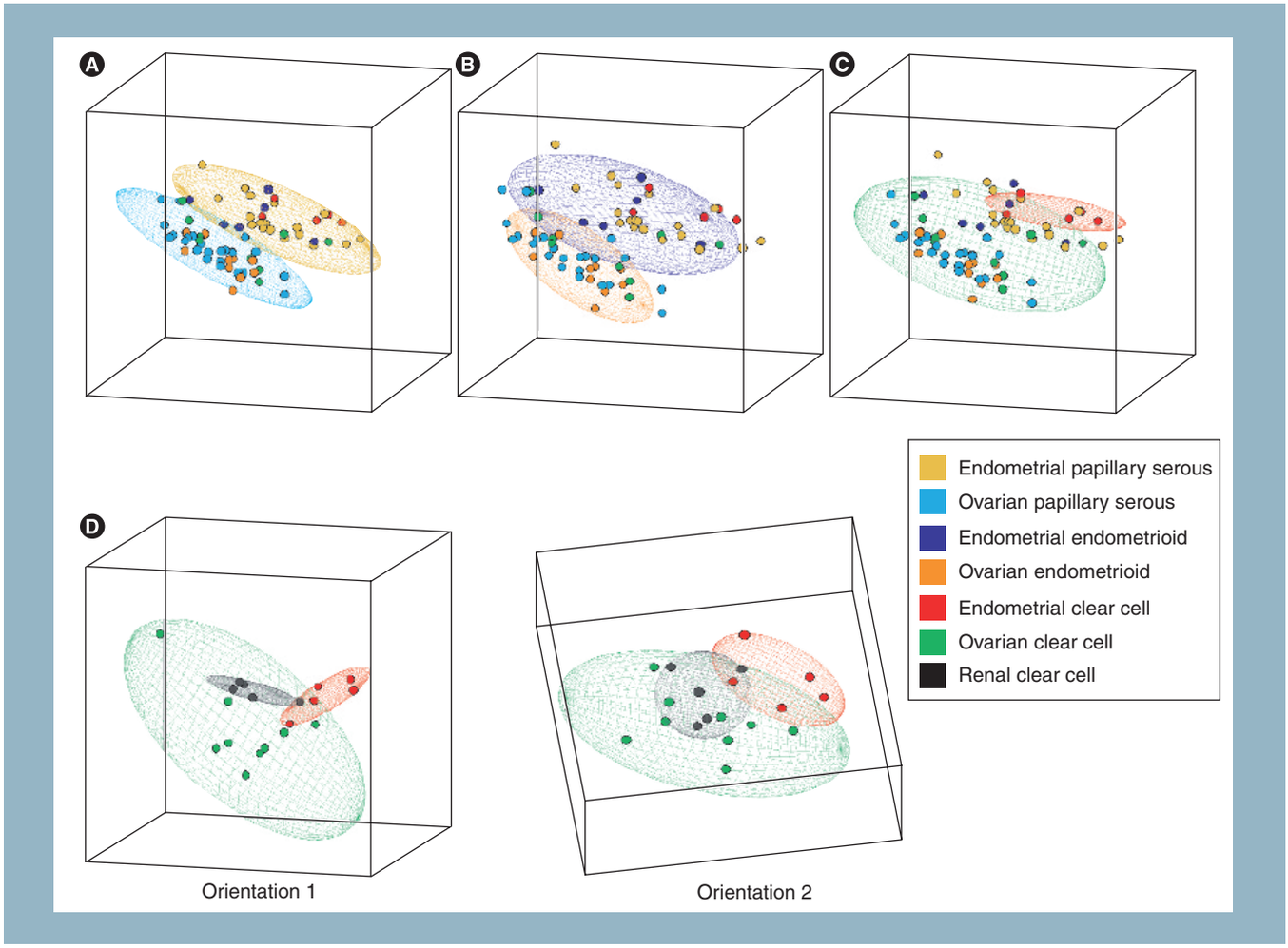

Figure 2. Gene-expression analysis of endometrial and ovarian tumors. (A) Nonoverlapping gene-expression profile of endometrial and ovarian papillary serous tumors, **(B)** nonoverlapping gene-expression profile of endometrial and ovarian endometrioid tumors, **(C)** overlapping gene-expression profiles of endometrial and ovarian clear-cell tumors, and **(D)** overlapping gene-expression profiles of endometrial, ovarian and renal clear-cell tumors.
Adapted with permission from [25].

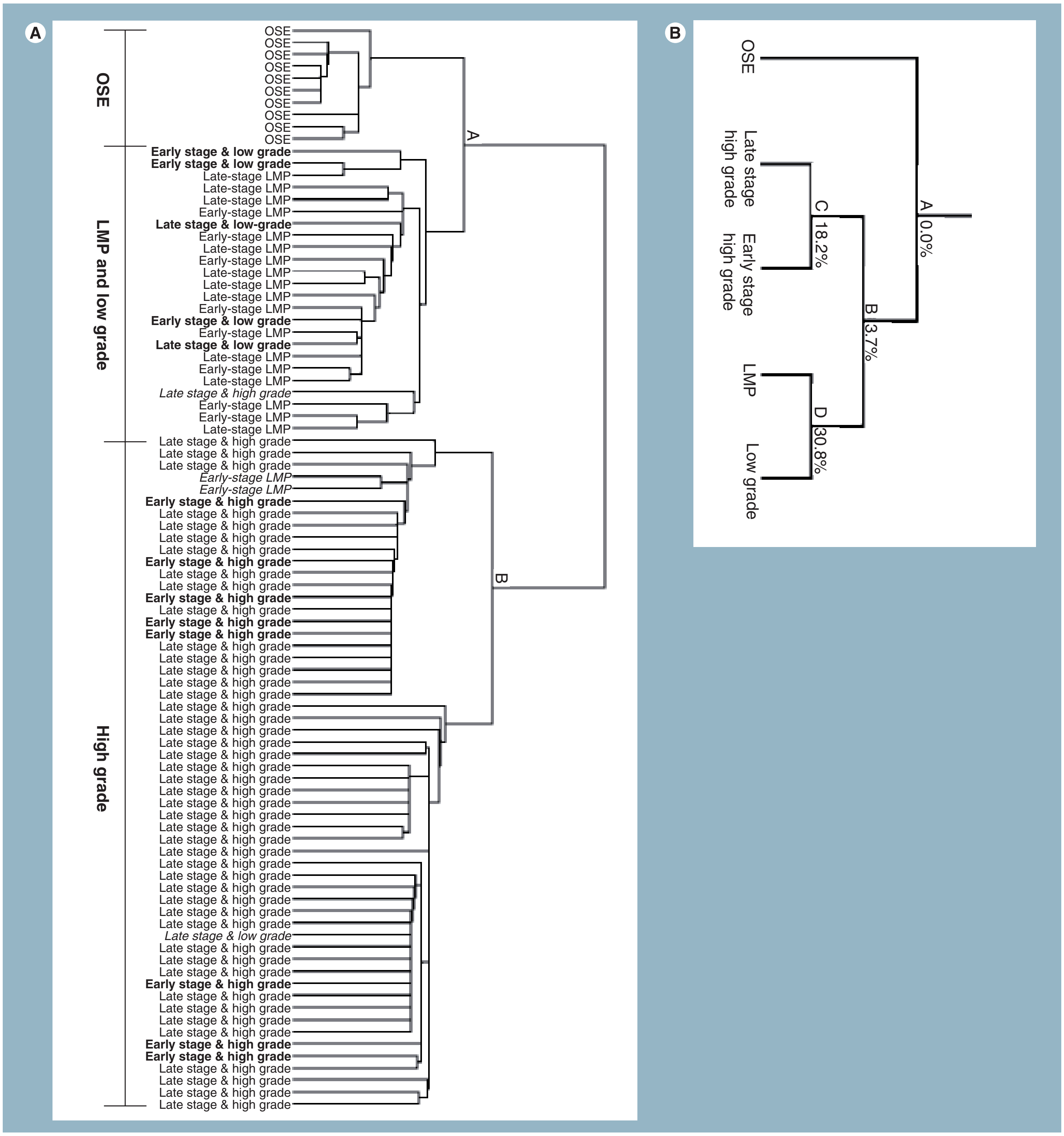

Figure 3. Genomic analysis separating low malignant potential and low-grade tumors from high-grade tumors. (A) LMP and low-grade tumors cluster closely together (node A) while late- and early-stage high-grade serous ovarian carcinomas associate closely in different clusters (node B). **(B)** Binary tree prediction assessing rate of misclassification in **(A)**. The misclassification rate of OSE versus tumor is 0.0% (node A), the misclassification rate of high-grade versus LMP and low-grade tumors is 3.7% (node B), the misclassification rate of late stage high grade versus early stage high grade is 18.2% (node C) and the misclassification rate of LMP versus low grade is 30.8% (node D). A high misclassification rate demonstrates the sharing of many coexpressed genes.
LMP: Low malignant potential; OSE: Ovarian serous epithelium.
Adapted with permission from [33].

The development of LMP and low-grade tumors certainly requires additional molecular events, specifically the activation of the RAF/RAS/MEK pathway. In 1993, Teneriello *et al.* found *K-RAS* mutations at codon 12 in six out of 20 (30%) borderline

tumors of mucinous or serous histology but not in serous or mucinous ovarian carcinomas [32]. In 2006, Mayr *et al.* analyzed more than 100 tumor samples and confirmed that *K-RAS* mutation was seen in borderline tumors, but these mutations were rarely seen in high-grade tumors [35]. Indeed, 11 out of 33 (33%) borderline mucinous and borderline serous tumors harbor *K-RAS* mutations at codon 12. Mayr *et al.* also reported that five out of 11 (31%) of borderline serous tumors harbor BRAF mutations at *V600E*. However, no high-grade serous carcinoma (0/38) contained a BRAF mutation [35].

The activation of the RAF/RAS/MEK pathway is linked to resistance to apoptosis and is involved in proliferation [36]. It has been shown that the use of AZD6244, a MEK-1/2 inhibitor, has antitumor activity in cells carrying *BRAF* and *RAS* mutations [37]. In stage IV melanoma patients with known *BRAF* mutations, treatment with AZD6244 resulted in prolonged remission [36]. Studies targeting *BRAF* mutations in ovarian cancer are ongoing and may yield promising results. GOG 239 is a Phase II trial of AZD6244 in recurrent low-grade tumors. The trial has completed patient accrual and is awaiting analysis.

Heterogeneity in high-grade tumors

Gene expression

High-grade tumors account for approximately 80% of all epithelial ovarian cancers with the vast majority classified as serous (92–97%) [38,39]. All serous high-grade tumors appear similar under the microscope. However, the clinical behavior of these tumors is quite variable in terms of response, rate of recurrence and patient survival [21], and recent genomic data have provided a molecular basis for this heterogeneity.

The use of genomics to identify subsets of patients with different prognosis is a powerful tool for individualizing treatments. With that aim, Tothill *et al.* identified subtypes within high-grade (2–3) serous tumors with distinct survival characteristics [34]. The four subtypes, from poorest survival are: high stromal response (C1); high immune response (C2); low stromal response (C4); and mesenchymal, low immune signature (C5). Spentzos *et al.* also identified a 115-gene signature termed the ovarian cancer prognostic profile that was a determinant of overall survival. Those in the unfavorable group had overexpression of genes such as the receptor protein tyrosine kinase, PDGF and VEGF-C [38,40].

A study by Mok *et al.* has demonstrated that, based on gene-expression profile, patients with serous high-grade tumors can be separated into two distinct survival groups: low risk and high risk [41]. Using Affymetrix U133 Plus 2.0 GeneChip® microarrays to analyze 53 advanced-stage, high-grade papillary serous primary tumors, the study identified and validated a gene profiling signature that is predictive of survival outcome for these patients. Interestingly, a subset of the high-grade serous tumors contained overexpression of MAGP2, which is involved in cell motility and survival. *MAGP2* ranked highest in Cox hazard ratio and was identified as an independent predictor for shorter survival. Furthermore, downstream effectors of MAGP2, such as *PXN, FAK, GRB2* and *SOS2*, were also overexpressed in a subset of high-grade serous tumors. This approach will undoubtedly be able to identify subsets of patients whose tumors are biologically unique and have pathways that can be targeted.

A different approach is to identify gene-expression patterns that predict response to chemotherapy. Recently, the 93-gene signature termed the chemotherapy response profile was identified by Spentzos *et al.* [39,40]. The study revealed overexpression of 77 genes (e.g., the antiapoptotic protein ICAM2) in the chemotherapy-resistant patients and overexpression of 16 genes (such as the proapoptotic protein BAX) in the chemotherapy-sensitive patients.

The molecular analysis mentioned previously can revolutionize the management of treatments for patients postcytoreductive surgery. Patients could be stratified based upon their gene signatures: patients with a good prognostic profile could receive standard therapy, while patients with a poor prognostic profile could be further tested for activation pathway that could be targeted by individualized therapy with or without the standard therapy [40].

Mutations

Although gene-expression signatures will provide valuable information for the appropriate stratification of patient groups, gene mutation and/or amplification can also assist in this process. Among high-grade tumors, *PIK3CA* is activated by mutation or amplification in approximately 30–40% of cases [42]. *PIK3CA* codes for the p110α, a catalytic subunit of PI3K, a family of signal transducers whose signaling cascades, through activation of AKT and mTOR, result in cell growth, proliferation, differentiation and survival [43–45]. Analyzing a set of 182 primary

ovarian tumors, Campbell *et al.* found that either a mutation or amplification of *PIK3CA* is present in 30.7% of malignant serous carcinomas but not in borderline tumors [42]. The study has also found that in malignant endometrioid and clear-cell tumors, the *PIK3CA* mutation or amplification rate is 45%. It has been demonstrated that mTOR inhibitors, such as rapamycin and its analogs, CCI-779 and RAD-001, can sensitize tumor cells to chemotherapy and radiation [45]. Using mTOR inhibitors in combination with chemotherapy to target tumors harboring *PIK3CA* mutations or amplifications may provide better survival outcomes for that subpopulation of high-grade ovarian cancer cases.

Another example of stratifying patients according to molecular events is that of BRCA1/2. In recent years, inhibition of poly-(ADP-ribose) polymerase (PARP) activity in patients with *BRCA1/2* mutations has generated much interest. BRCA1 and BRCA2 function in homologous repair of double-strand breaks, and cancer cells lacking that repair function have been successfully targeted with PARP inhibitors. In 1999, Moynahan *et al.* demonstrated that cells lacking functional BRCA1 were impaired in homology-directed repair of chromosomal double-strand breaks and suggested that nonhomologous repair had increased, perhaps in a compensatory manner [46]. In 2001, Moynahan *et al.* reported that cells lacking functional BRCA2 were also impaired in homology-directed repair of double-strand chromosomal breaks [47]. Therefore, a novel concept emerged, suggesting that cells defective in double-strand break homologous repair depend on alternative but less faithfully accurate DNA repair pathways, such as nonhomologous-end joining or single-strand annealing [48]. Farmer *et al.* have demonstrated that cells with nonfunctional homologous repair become sensitive to PARP inhibition [49]. According to the study, as PARP is involved in single-strand break repair, loss of PARP function leads to persistent single-strand breaks that then lead to double-strand breaks and multiple chromatid aberrations, resulting in loss of viability. In the same year, Bryant *et al.* demonstrated that PARP inhibition killed BRCA2-deficient tumors in mice [50].

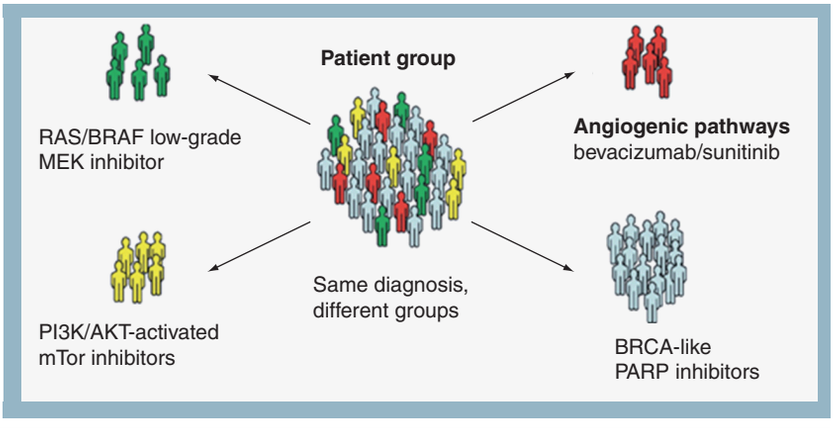

Figure 4. The promise of genotype-directed therapy for ovarian cancer patients. Molecular signatures specific to ovarian cancer subtypes can be targeted to improve overall survival.

Mutations in *BRCA1/2* or in the pathway for the inactivation of BRCA1/2 are common in high-grade serous carcinomas of the ovaries. Press *et al.* analyzed 49 ovarian carcinomas and reported that 26 out of 49 tumors (53%) had either *BRCA1* or *BRCA2* mutations, epigenetic loss of *BRCA1*, or were 'equivocal for *BRCA1* loss'. All 26 of the samples with *BRCA1* or *BRCA2* mutations were of high-grade serous or undifferentiated histology, which represented 68% of serous samples (26/38) [51]. Further analysis revealed that among the 38 high-grade serous or undifferentiated tumors, 47% harbored *BRCA1* mutations or epigenetic loss of *BRCA1*, 8% harbored *BRCA2* mutations and 13% were 'equivocal for *BRCA1* loss'.

Interestingly, the study has also reported that among those with loss of *BRCA1* through epigenetic events, 88% had increased copy numbers of PIK3CA and 89% had elevated expression levels of p53. In addition, it has been demonstrated by Altiok *et al.* that constitutively active PI3K can induce phosphorylation of BRCA1 in breast cancer cells, which has been suggested to alter the function of BRCA1 [52]. Furthermore, it has also been reported that high mRNA expression levels of both *BRCA1* and *ERCC1* in sporadic ovarian cancer are correlated with shorter overall survival (33 vs 46 months; $p = 0.04$); however, the data are preliminary [53].

There is much excitement over the use of PARP-1 inhibitors currently in Phase I and II clinical trials as a single agent (KU-0059436/AZD2281/olaparib for breast and ovarian cancer) and in combination with conventional cytotoxic agents (AG014699 with temozolomide in advanced solid tumors and Ino-1001 with temozolomide for malignant glioma) [54]. Other PARP-1 inhibitors currently in clinical trials for ovarian cancer include AG014699 as a single agent, AZD2281 with carboplatin, BSI-201 with carboplatin and gemcitabine, ABT-888 with topotecan, ABT-888 with carboplatin, paclitaxel, bevacizumab, ABT-888 and temozolomide.

Future perspective

Personalized approaches to cancer patients have been demonstrated to be effective for some cancers, such as lung and breast cancers. This has required the identification and validation of subsets of patients who have a distinct molecular feature that predicts for response to therapy. These efforts have involved the analysis of carefully annotated tumor specimens and large prospective trial validation of the genomic biomarkers. For ovarian cancer, the specimens to test this have only recently become available, and major efforts have just recently begun to integrate the genomic data into clinical trials.

In recent years, there has been recognition that the standard treatment regimen available for ovarian cancer is incongruous with the heterogeneity of the ovarian cancer histology subtypes and grades. The use of novel biologics targeting molecular pathways in the tumorigenesis of ovarian cancer is a viable option, but the subpopulation that will benefit most from each biologic must be identified. Treatment regimens should be tailored to each patient based on the molecular profiles associated with the grade and histology of the debulked ovarian tumors (Figure 4). Adjuvant chemotherapy comprised of carboplatin and paclitaxel in combination with a PARP inhibitor, mTOR inhibitor, MEK-1/2 inhibitor or an antiangiogenic followed by targeted, single-agent consolidation therapy may help prolong remission. To improve the amount of meaningful data and verify the effectiveness of novel biologics, Phase II/III clinical trials must distinguish patient populations not only by grades (LMP and low grade vs high grade) but also by histology (papillary serous vs clear-cell tumors) and gene-expression signatures outlining survival prognosis, chemosensitivity and activated pathways. Improving overall survival for ovarian cancer patients depends on the development of molecular tools to help streamline the analysis of recently debulked tumors and identification of patient-specific molecular targets for individualized therapeutic regimens.

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Executive summary

Tumor heterogeneity mediates the value of individualized therapies

- Benefits of personalized therapy have been demonstrated in breast and non-small-cell lung cancer.
- Use of EGF receptor tyrosine kinase inhibitors with standard chemotherapy for non-small-cell lung cancer demonstrated benefit to the nonsmoking patient subpopulations over standard care alone.
- Breast tumor gene profile provides an effective prognostic tool to determine which patients require standard adjuvant chemotherapy, and which do not.

Heterogeneity in ovarian cancer: histology

- Ovarian tumors can be categorized based on numerous histology types.
- Significant differences exist between histology types regarding stage at first diagnosis and overall prognosis.
- These are broad molecular differences between clear-cell and nonclear-cell ovarian tumors.

Heterogeneity in ovarian cancer: grade

- Low malignant potential (LMP) and low-grade tumors are distinctly different from high-grade tumors.
- LMP tumors of the ovary possess morphologic features of malignancy but have a much higher 5-year survival prognosis than high-grade tumors.
- Dysregulation of genes that activate the wild-type p53 pathway have been found in low-grade and LMP tumors.
- Mutations in *BRAF* or *KRAS* are common among low-grade and LMP tumors.
- *PIK3CA* mutations or amplifications are prevalent among high-grade tumors.
- Mutations in *BRCA1* or *2* are also indicative of high-grade papillary serous tumors.
- *MAGP2* has been identified as an independent predictor for shorter survival in high-grade cancer cases.
- Gene-expression profiles subdivide high-grade serous cases by chemosensitivity and survival.

Future perspective

- Adjuvant chemotherapy comprised of carboplatin and paclitaxel in combination with a poly(ADP-ribose) polymerase inhibitor, mTOR inhibitor, MEK-1/2 inhibitor or an antiangiogenic followed by targeted, single-agent consolidation therapy may help prolong remission.
- Development and application of molecular tools will help to streamline the identification of patient-specific molecular targets.

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