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Molecular staging of gynaecological cancer – what is the future?

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Abstract

The purpose of cancer staging is to classify cancers into prognostic groups and to allow for comparison of treatment results and survival between patients and institutions. Staging for gynecologic cancers is based on extent of disease and metastasis, which was historically determined by physical examination and is now based on surgical and histologic examination of tumor specimens. While extent of disease is currently considered the most important predictor of recurrence and survival, current staging does not include molecular features that are associated with tumor aggressiveness, response to therapy, and prognosis. This review focuses on genomic and proteomic features of gynecologic cancers and the future of biomarkers in staging classification.

Keywords

Gynecologic cancer; biomarker; molecular classification; staging; prognosis

Introduction

Historically, gynecologic cancers were clinically staged and the extent of disease was predicted based on physical examination and radiographic findings. Given that clinical examination was inaccurate in determining lymph node involvement, distant metastasis, histologic grade, and other prognostic pathologic features, surgical staging was adopted for all but cervical cancer. The late 1980s International Federation of Gynecology and Obstetrics (FIGO) staging nomenclature for carcinoma of the ovary, endometrium and vulva became dependent on surgical and pathologic findings.

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Current cancer research focuses on understanding the molecular and mutational events that occur in early carcinogenesis and lead to metastasis. Sequencing, microarray and proteomic methods allow analysis of all genes of a tumor at the DNA, RNA and protein levels, but we must determine which alterations are clinically significant. In the last decade, researchers have begun performing molecular sub-classification of several cancers [1-3], although there is no clear consensus of which prognostic markers to include in staging. None of the gynecologic cancers are staged with molecular markers, but it is imperative that we consider the use of newly available technologies to identify markers that are truly prognostic and predictive of survival, recurrence and treatment response.

I. Uterine carcinoma

In addition to FIGO stage, assessments of tumor grade, lymphovascular space invasion (LVSI), and histologic subtype are important in the prognosis and management of endometrial cancer (EC) [4]. In 1983, Bokhman described two subtypes of EC based on different clinical observations, but now type I includes grade 1 and 2 endometrioid endometrial carcinomas (EEC) and type II includes grade 3 EEC and non-EEC (serous, clear cell and undifferentiated) [5]. Grade and cell type are distinguished by microscopy based on morphologic features, but this method often lacks reproducibility [6] and differences in grade and histology are better reflected in their genetic and molecular profiles [7]. The progression from normal endometrium to EEC or non-EEC may not always follow a linear pathway and non-EEC may develop from high-grade EEC after further molecular alterations and tumor progression [8]. Tumors with mixed features may represent this progression and histology diagnosis by microscopy may be difficult. Thus, improved classification systems are needed for EC.

A. Markers associated with histology

Molecular profiling can distinguish EEC from non-EEC. EECs have a higher frequency of *PTEN*, *PIK3CA*, *CTNNB4*, *KRAS*, *FGFR-2* and *ARID1A* mutations, estrogen and progesterone receptor (ER/PR) expression, and microsatellite instability (MSI). High-grade EECs have higher frequencies of *PIK3CA* and *p53* mutations than low-grade EECs.

Uterine serous carcinomas (USCs) exhibit a high frequency of mutations in *p53*, *HER2*, *PIK3CA* and *PPP2R1A*, loss of function in p16 and E-cadherin, overexpression of Stathmin and Cyclin D1/E, and chromosome instability. Clear cell carcinomas show mutations in *PIK3CA*, *PTEN* and *ARID1A*, loss of function of BAF250a (ARID1A protein), and positive immunoreaction for HNF1 β [8, 9]. There are no clear guidelines for the use of immunohistochemistry (IHC) and molecular genetic profiling in cell type classification, but they often represent an improvement over the current histopathologic assessment. For example, McConechy et al. were able to accurately reclassify diagnostically difficult cases into correct histologic subtype based on exome-sequencing of nine genes [10]. Similarly, molecular characterization of 373 ECs by the Cancer Genome Atlas (TCGA) Research Network helped reclassify 25% of high-grade EECs into USCs [11]. These findings demonstrate that systematic immunoprofile and mutation analysis for certain biomarkers can improve tumor classification and prognostication.

B. Prognostic markers and targeted therapies

Numerous mutations and biomarkers are reported to have prognostic roles in large EC studies [9], but it is neither practical nor cost-effective to incorporate them all into molecular staging. When interpreting biomarker studies, special attention should be given to the techniques used in detecting alterations and the statistical analysis performed to show prognostic capabilities. Ideal biomarkers will be easy and economical to evaluate and will have prognostic value after adjusting for known clinical and pathologic prognostic factors [12].

PI3K-AKT-mTOR pathway

PTEN: This tumor suppressor gene encodes a phosphatase that enables cell cycle arrest and apoptosis by antagonizing the PI3K-AKT pathway [13]. *PTEN* mutations are present in 56-80% of low-grade EEC, 40% of mixed carcinomas, and are nearly absent in non-EEC [8, 9]. They are associated with a more favorable histology and outcome. However, the independent prognostic value of *PTEN* needs further evaluation. Early studies showed that *PTEN* mutations are associated with early FIGO stage and prolonged survival [14], but this favorable outcome may be limited to mutations not involving exons five through seven [15]. In a population-based study, loss of PTEN expression as a result of promoter methylation was associated with advanced stage [16]. While *PTEN* mutations are associated with EEC, these mutations may represent a high-risk group within EECs and adjuvant therapy could be considered in these patients.

PIK3CA: Activating mutations in the oncogene *PIK3CA* leads to up-regulation of the anti-apoptotic PI3K-AKT pathway. Such mutations are present in 44% of mixed histotype ECs, 28% of EECs, and 21% of non-EECs [8, 9]. Catus et al. suggested that mutations in exon 20 are important in the progression of low-grade to high-grade EEC and in the pathogenesis of non-EEC [8]. With this biomarker, the specific sequence alteration carries prognostic significance and should be reported with mutation results.

Targeted Therapies: Drugs targeting the PI3K/AKT/mTOR pathway are currently in pre-clinical and clinical trials [17]. Results of phase II trials evaluating mTOR inhibitors in the treatment of recurrent or metastatic EC have been modest. Temsirolimus was evaluated in a phase II trial of chemotherapy-naïve patients and patients who received one prior chemotherapy regimen [17]. Primary treatment with temsirolimus produced a 14% radiographically confirmed partial response rate (PRR) and a 69% stable disease rate (SDR) after median follow-up times of 5.1 and 9.7 months respectively. Temsirolimus produced a 2% PRR, 48% SDR, and 48% progressive disease (PD) rate in recurrent EC. Half the patients required a dose reduction. Response was not associated with *PTEN* mutations, loss of PTEN expression or immuno-response for downstream targets of AKT, mTOR and S6. Currently, a randomized phase II trial is evaluating temsirolimus with carboplatin and paclitaxel after promising results from a phase I trial (NCT00977574).

A phase II trial of ridaforolimus produced an 11% PR rate and 18% SD rate after 4 months of therapy in 45 patients with advanced EC [17]. Everolimus treatment in recurrent or metastatic EC patients showed no RR, and half the patients with SD at first follow-up

discontinued the drug due to toxicities while the other half developed PD [18]. Pre-clinical trials of AZD8055 (a dual mTOR 1/2 inhibitor) and GDC-0980 (a dual PI3K and mTOR 1/2 inhibitor) show promise in USC cell lines and clinical trials are anticipated [19, 20].

KRAS-MAPK pathway

KRAS: This oncogene encodes a GTPase that transduces signals from growth receptors on the cell surface to the nucleus leading to transcription and translation via the mitogen-activated protein kinase (MAPK) pathway [8]. KRAS also binds to PIK3CA and activates the PI3K-AKT-mTOR pathway. *KRAS* mutations are found in 10-30% of ECs and are more common in low-grade EECs. In a study with 466 EECs, *KRAS* mutations were identified in 19% of samples and were associated with longer progression-free survival (PFS) on univariate and multivariate analysis [21]. However, pre-clinical studies show that *KRAS* mutations predicted resistance to single-agent therapy targeting the PI3K-AKT-mTOR pathway and cell lines without *KRAS* alterations had improved sensitivity to NVP-BEZ235 (a dual PI3K/mTOR inhibitor) and everolimus [22].

Targeted therapies: MEK inhibitors inhibit key enzymes in the MAPK pathway and have been studied in combination with PI3K-AKT-mTOR inhibitors in pre-clinical trials. Addition of MEK inhibitor PD98059 improved the sensitivity of *KRAS* mutant EC cell lines to NVP-BEZ235 and everolimus [22]. Single-agent GDC-0941 (PI3K inhibitor) controlled tumor growth in xenografted mice, but combination with MEK inhibitor PD0325901 led to significant reduction in tumor size [23]. A randomized phase II trial is evaluating MEK inhibitor trametinib with and without AKT inhibitor GSK2141795 in patients with persistent or recurrent EC (NCT01935973).

WNT/ β -catenin signaling pathway

CTNNB1: This Wnt family member encodes the β -catenin protein that acts as a transcription factor [8]. Phosphorylation of amino acids encoded by exon 3 of *CTNNB1* leads to degradation of β -catenin. Mutations in this region prohibit β -catenin degradation and eventually lead to cell proliferation and progression to pre-cancerous and cancerous phenotypes due to unchecked transcription activation. *CTNNB1* mutations are present in 14-44% of EECs but are absent in non-EEC and mixed tumors [8]. Thus the prognostic value of mutation status should be studied in large cohorts of EECs. Mutation and expression analysis of 192 EECs showed that tumors with *CTNNB1* exon 3 mutations led to Wnt/ β -catenin pathway activation and were associated with low-grade cancers, early stage disease, younger patients and shorter survival even after adjusting for age, grade and stage of disease [24]. *CTNNB1* mutations that did cause activation of the Wnt/ β -catenin pathway were not associated with poor survival. Therefore, the presence of *CTNNB1* exon 3 mutations in young patients with early stage and low-grade EEC may be a poor prognostic feature and should prompt closer follow-up and strong considerations for treatment and possibly adjuvant therapy.

P53 alterations—The tumor suppressor *p53* encodes a transcription factor that inhibits cell growth and promotes cell-cycle arrest and apoptosis in response to DNA damage. *P53* loss leads to chromosomal instability, aneuploidy and inhibition of apoptosis [25]. *P53*

mutations occur in 60-85% of non-EEC and are associated with aggressive histology, advanced stage and poor clinical outcome [8]. In 131 surgically staged EC patients, 30% had *p53* mutations and an 11-fold increased risk of death compared to patients without *p53* mutations, after adjusting for histology, grade, FIGO stage, and lymph node metastasis [26]. A multivariate analysis for *p53* mutations after stratifying for adjuvant radiotherapy and found lower survival rates in patients with mutations [27]. Adjuvant radiotherapy improved OS of *p53* mutation carriers to match that of patients without *p53* mutations, but did not improve OS in patients without mutations.

P53 mutations are also seen in 2% of low-grade EECs, 20% of high-grade EECs, 54% of mixed carcinomas, 75% of endometrial intraepithelial carcinomas, and up to 90% of USCs [8, 28, 29]. Simultaneous loss of *p53* and activating *PIK3CA* mutations in high-grade and mixed EECs promotes aggressive malignant transformation and leads to poorer survival than in patients with *p53* mutations alone [30]. These studies suggest that *p53* mutations may influence early events in serous carcinogenesis and induce progression of EECs to non-EECs. Therefore, detecting *p53* mutations and PI3K-AKT-mTOR pathway activations is critical in identifying women at high risk of poor outcomes and treating them appropriately.

Receptor Tyrosine Kinases (RTKs)

EGFR (Erb-B1): Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein belonging to the ErbB family of RTKs. Receptor activation initiates a complex set of pathways including PI3K-AKT-mTOR and KRAS-MAPK causing cell growth and proliferation [17]. EGFR overexpression is reported in 46% of EECs and 34% of non-EECs [9]. The prognostic value of EGFR overexpression is debatable: some studies show an association with poor survival, but others demonstrate no significant prediction of outcome [31].

Targeted therapies: EGFR inhibitors gefitinib and erlotinib have not shown a clinically significant RR or benefit in PFS and OS in phase II trials of advanced and recurrent EC [31]. Furthermore, neither trial showed an association between response and EGFR overexpression.

HER2 (Erb-B2): This oncogene encodes human EGFR-2. HER2 protein overexpression seen in 20-40% of ECs, with highest frequency in non-EECs (43%) then high-grade EECs (29%) then low-grade EEC (10%) is an independent predictor of poor OS in EC [8]. HER2 overexpression negatively affects PFS and OS in ECs and HER2 expression may predict sensitivity to paclitaxel and therapies targeting the PI3K-AKT pathway [32]. For example, high HER2 expression in USCs was associated with increased sensitivity to AZD8055 and GDC-0980 [19, 20]. *HER2* amplification and overexpression are capable of predicting survival outcomes and response to targeted therapies, therefore genetic and IHC analysis should be performed for comprehensive counseling and individualized targeted therapy recommendations.

Targeted therapies: Monoclonal antibodies targeting the extracellular domain of HER2 receptors such as trastuzumab and pertuzumab are approved for use in HER2-positive breast

cancer. A phase II trial of 33 patients with HER2 overexpressing advanced or recurrent EC treated with trastuzumab did not lead to a tumor response, but 36% had SD and 55% had PD [33]. A randomized phase II trial is evaluating trastuzumab in combination with carboplatin and paclitaxel in HER overexpressing USCs (NCT01367002).

Treatment of persistent or recurrent EC with lapatinib, a dual EGFR/HER2 inhibitor, showed a 3% PR, 23% SD and 70% PD rate with median PFS of 1.8 months in all groups. Mutation analysis in this study identified three new *EGFR* mutations, one of which (E690K, in exon 18) was associated with partial response [34]. Detecting this mutation in patients with EGFR overexpressing ECs may predict benefit with lapatinib.

FGFR2: Activating mutations or amplifications in oncogene *Fibroblast growth factor receptor-2* are present in 10-16% of EECs [21, 31]. Mutations in *FGFR2* were almost mutually exclusive of *KRAS* mutations, but they individually occurred in tumors with MSI. *FGFR2* mutations were more common in low-grade tumors and were associated with shorter OS and disease-free survival in stage I and II disease [21].

Targeted therapies: *FGFR2* mutations are associated with chemoresistance and therefore FGFR2 inhibition may improve response to chemotherapy. Pre-clinical studies of EC cell lines with *FGFR2* mutations showed that knockdown or inhibition of FGFR2 with PD173074 resulted in cell cycle arrest and apoptosis [31]. Combination of PD173074 and cytotoxic chemotherapy showed synergistic activity with doxorubicin or paclitaxel [35]. Brivanib and nintedanib are RTK inhibitors that block FGFR and vascular endothelial growth factor receptor (VEGFR). Phase II trials of these drugs in recurrent or persistent EC showed 19% RR with 30% 6 month PFS for brivanib and 9% PRR with 22% 6 month PFS for nintedanib [36, 37].

VEGFR: Ligand binding and activation of VEGFR leads to endothelial proliferation, angiogenesis, and increased tumor growth [31].

Targeted therapies: The monoclonal antibody bevacizumab binds and inactivates VEGF, thereby inhibiting endothelial and possibly tumor proliferation. A phase II trial evaluating its efficacy in recurrent or persistent EC showed a RR of 14%, median PFS of 4.2 months and OS of 10.5 months [38]. Patients treated with a combination of bevacizumab and temsirolimus had a 25% RR and median OS of 16.9 months, but combination was significantly more toxic than bevacizumab alone [39]. The safety and efficacy of adding bevacizumab to carboplatin and paclitaxel in advanced EC patients is being evaluated in a phase II open-label clinical trial (NCT00513786).

Hormone Receptors

ER/PR: Estrogen and progesterone receptor positivity are associated with endometrioid histology and significantly improved PFS [40].

Targeted therapies: Median PFS after medroxyprogesterone, anastrozole or arzoxifene use in recurrent or advanced EC ranges from 1 to 3.7 months [12]. The ER antagonist fulvestrant produced a 17% RR and 10-month median PFS in patients with ER positive recurrent or

metastatic EC [41]. For stage I EC, a systematic review reported no benefit of adjuvant hormone therapy in 7 of 9 randomized controlled trials [42]. Nevertheless, IHC of ER/PR is a good and inexpensive biomarker and may predict response to non-toxic hormone therapy.

DNA Polymerase E (*POLE*) mutations—Based on genomic and proteomic characterization of 373 ECs, TCGA research network identified the *POLE* ultramutated group in which no recurrences occurred. Although these results seem like a breakthrough in association between molecular profiles and clinical prognosis, the data should be interpreted keeping established histopathologic prognostic factors in mind. *POLE* mutations were present in 10% of EEC and absent in non-EECs. Given the association of *POLE* mutations with low-grade EECs, we would expect a better clinical outcome compared to serous and high-grade EEC tumors. Billingsley et al. identified *POLE* mutations in 5.6% of 535 EECs. There was no association between *POLE* mutation and PFS (univariate analysis) or OS (multivariate analysis), but only one patient with *POLE* mutation recurred [43]. Another study looking specifically at grade 3 EEC showed an improved PFS, and no recurrence for patients with *POLE* mutant tumors [44]. *POLE* shows promise as a reliable marker but needs better characterization in the context of EEC.

C. Conclusions

Characterization of ECs based on high-throughput data from molecular profiles is already underway. The four categories described by TCGA have significant overlap with current histologic subtypes. Pronounced differences in mutation profiles of low-grade EEC, high-grade EEC, and non-EECs strengthens the hypothesis that these histologic subtypes are probably more heterogeneous than originally thought. Correct classification is essential to predict tumor behavior, risk of recurrence and response to adjuvant and targeted therapies. Reliable and valid classification of EC on the basis of molecular profiling may result in improved patient counseling, choice of adjuvant therapy, and clinical outcomes.

II. Ovarian carcinoma

The two most important prognostic factors for ovarian cancer (OC) are FIGO stage and the presence of macroscopic residual tumor after primary surgical debulking [45]. For decades, all OCs were treated the same with aggressive surgical resection followed by platinum chemotherapy. Based on morphologic and genetic characteristics, two distinct pathways for ovarian carcinogenesis were proposed [46]. Type I OCs are slow-growing, arise from precancerous lesions, and are associated with *KRAS* and *BRAF* mutations. Type II OCs are aggressive, arise de novo, present with metastases, and are associated with *p53* mutations. Similar to EC, research shows this classification may be too simplistic as tumors within each subtype differ in carcinogenesis events, patterns of spread, precursor lesions, response to chemotherapy and clinical outcomes. Now, OC is classified into five subtypes including high-grade serous (HGSOC), low-grade serous (LGSOC), endometrioid (EOC), clear-cell (CCC), and mucinous (MC) carcinomas [47]. In a study of 575 optimally resected OC patients, histology was associated with outcome [48]. Specifically, patients with serous histology had a shorter OS than patients with EOC and MC. Historically, diagnosis was

based on morphological features, but IHC to detect biomarkers is more useful in distinguishing tumor behavior.

A. Markers associated with histology

IHC for cytokeratin-7 and PAX-8 can be performed to distinguish mullerian from other primaries and IHC for WT-1 can distinguish between serous carcinomas of ovarian versus endometrial origin [47]. Serous carcinomas are WT-1 and ER positive, OEC are ER positive and WT-1 negative, CCC are HNF1 β positive and negative for WT-1 and ER, and MC are ER and CA-125 positive and can be negative or weakly positive for gastro-intestinal markers like CEA, CA 19-9 and CDX2 [47]. High nuclear Ki-67 expression is seen in HGSC but absent in LGSC.

Like EC, histologic subtypes of OC have characteristic genetic profiles. HGSCs are characterized by mutations in *p53*, *BRCA1-2*, and aneuploidy, whereas LGSCs have *KRAS* and *BRAF* mutations. EOCs have mutations in *ARID1A*, *CTNNB1* and *PTEN* along with MSI whereas CCCs carry mutations in *ARID1A*, *PIK3CA*, *PTEN* and *KRAS* [47]. Screening for these biomarkers is not a part of OC classification.

B. Markers associated with cancer stem cells

Despite good response rates to cytotoxic chemotherapy, the recurrence rate for OC remains high and may be due to the presence of cancer stem cells (CSCs) in the original tumor. Although they represent a small proportion of the tumor, CSCs are believed to be resistant to chemotherapy and can grow rapidly, thereby repopulating chemotherapy resistant recurrences [49]. Markers for CSCs in breast, hematopoietic and brain cancers including CD44, CD117, and CD133 are being studied in OC.

CD44 is a hyaluronate receptor seen in breast CSCs and CD44+ cancer cells have been isolated from the primary tumor, metastatic sites and ascites of OC patients. These cells were chemoresistant and able to generate new heterogeneous (CD44+/CD44-) tumors when transplanted into immune-compromised mice [50]. More than 25% of CD44+ cells in ascites fluid was predictive of a higher recurrence rate and a shorter median PFS in 19 consecutive advanced-stage OC patients [51].

CD117 is a stem cell growth factor receptor encoded by proto-oncogene *KIT*. It is an RTK and may play a role in carcinogenesis and chemoresistance by activation of the WNT/ β -catenin pathway [52]. In a series of 25 OC patients, 40% of tumors were positive for CD117 expression by IHC and were associated with chemo-resistance [53].

CD133 is a transmembrane glycoprotein expressed on CSCs in acute myeloid leukemia and brain cancers. CD133+ OC cells were chemoresistant and tumorigenic generating tumors with CD133+ and CD133- cells [54]. In a study of 400 OC samples, 31% were positive for CD133 expression and these cancers were associated with high-grade serous histology, advanced stage, and non-response to chemotherapy [55]. Multivariate analysis showed that CD133 expression was an independent predictor of shorter recurrence-free survival (RFS).

Targeted therapies—Trials of CD44 ligands conjugated to cisplatin and paclitaxel in intraperitoneal OC murine models have shown decreased growth and weights of tumor [56]. Imatinib, a KIT tyrosine kinase, showed disappointing results in the treatment of recurrent platinum-resistant LGSOC with no RR, one of 11 patients with SD for 7.3 months and the rest with PD [56].

A high percentage of CSCs in primary tumors is associated with resistance to chemotherapy and shorter RFS. Evaluating such patients more often may lead to early diagnosis of recurrence, but it is unclear whether early diagnosis and treatment would affect response or survival. Nonetheless, the presence and percentage of CSC markers may be an effective screening tool to diagnose recurrence.

C. Prognostic markers and targeted therapies

BRCA1 and BRCA2—These tumor suppressor genes encode proteins involved in homologous recombination repair of damaged DNA. Germline mutations in these genes lead to a hereditary breast and ovarian cancer syndrome with a 30-70% lifetime risk of developing ovarian cancer, mostly HGSOC type [57]. Somatic mutations in *BRCA1-2* and hypermethylation of the *BRCA1* promoter also cause aberrations in the BRCA pathway. Up to 50% of HGSOCs have molecular alterations in the BRCA pathway [58]. *BRCA1* mutations are associated with longer median survival than matched controls without a *BRCA* mutation [59]. OCs with *BRCA2* mutations are associated with higher chemotherapy RR and longer PFS and OS than patients without *BRCA* mutations [60]. Most recently, 390 OC samples were assessed for germline and somatic mutations in 13 genes involved in the homologous recombination pathway. Mutation rate in non-serous OC (28%) was similar to the rate in serous carcinomas (31%). Mutations in the homologous recombination pathway predicted response to platinum-based chemotherapy and were associated with longer OS [61]. *BRCA* mutation status is now routinely evaluated in patients with ovarian, fallopian tube or primary peritoneal cancer [62].

Targeted therapies: Poly (ADP-ribose) polymerase (PARP) is involved in base-excision repair after single-stranded DNA breaks. PARP inhibitors induce double-strand DNA breaks, and cells that are lacking the homologous recombination repair pathways become genomically unstable and die. Three such inhibitors, Olaparib, Rucaparib and Veliparib are being evaluated in the treatment of *BRCA* mutation-positive OCs [63]. Olaparib showed an insignificantly longer PFS and significantly lower toxicities than pegylated liposomal doxorubicin in platinum-resistant OC [63]. When used as maintenance oral therapy after platinum-based chemotherapy in recurrent OC, olaparib demonstrated better PFS than placebo and it is now being evaluated as maintenance therapy after primary platinum-based adjuvant therapy in a phase III trial (NCT01844986).

Most promisingly, the addition of cediranib to olaparib resulted in prolongation of median PFS from 9 to 17.7 months in recurrent OC [63]. This regimen was active in patients with and without BRCA mutations. Another similar clinical trial evaluating the combination of carboplatin, paclitaxel, bevacizumab and veliparib in newly diagnosed stage II-IV ovarian, fallopian tube and primary peritoneal carcinoma was designed (NCT00989651). Other

combination regimens of interest include PARP inhibitors and PI3K inhibitors. A phase I trial combining PI3K inhibitor BKM 120 and olaparib showed antitumor response in recurrent HGSOC and triple-negative breast cancer [63].

KRAS/MAPK Pathway—Mutations in *KRAS* and *BRAF* activate the MAPK pathway and are present in 19% and 38% of LGSOC respectively [64]. *KRAS* mutations are also detected in 85% of MCs [65]. LGSOCs are less aggressive than HGSOCs, but are relatively non-responsive to conventional chemotherapy [66]. Prognosis after primary surgical resection is favorable and isolated recurrences can be managed with surgical excision, but targeted therapies are sought for surgically challenging cases.

Targeted therapies: In a phase II trial of recurrent LGSOC, Selumetinib had a 15% overall RR and 63% 6-months PFS rate [67]. An international phase III trial comparing MEK 162 to physician choice of chemotherapy in persistent or recurrent LGSC is currently active (NCT01849874). Trials combining MEK inhibitors and PI3K inhibitors are also being designed.

Tumor-infiltrating lymphocytes—A meta-analysis of the prognostic value of tumor-infiltrating lymphocytes (TILs) in OC showed an association with improved OS, but most studies looked at all epithelial OCs and did not adjust for histology [68]. A retrospective study of 500 OC samples evaluated the role of CD8+ T-cells in different histologic subtypes [69]. CD8+ cells were associated with improved disease-specific survival (DSS) in serous and MCs but not EOC and CCCs. However, failure to show prognostic value in EOC and CCC may be due to smaller number of cases and a better baseline OS in this group. After adjusting for stage and tumor cell types, the presence of CD8+ cells was predictive of DSS.

Targeted therapies: Ipilimumab (anti-cytotoxic T lymphocyte-associated antigen-4) and Nivolumab (a programmed cell death ligand-1 inhibitor) suppress immune inhibitory signals and lead to an anti-tumor T-cell response. These agents have been studied in small series of recurrent OC patients with impressive anti-tumor activity in some individuals [70, 71]. Other vaccines strategies targeting tumor-specific antigens are now being developed.

Receptor Tyrosine Kinases and Angiogenesis markers—Multiple proangiogenic growth factors, including VEGF, FGF, endothelial growth factor (EGF) and platelet-derived growth factor (PDGF) are secreted from endothelial and stromal cells and this process is of particular interest in ovarian cancer due to hematogenous spread and metastasis. Like EC, these biomarkers are correlated with prognosis but they do not predict response to anti-angiogenic agents [72]

Targeted therapies: Current anti-vascular strategies include drugs that target growth factors or inhibit the RTKs. Multi-receptor tyrosine kinase inhibitors pazopanib and nintedanib showed modest improvement in PFS after primary OC maintenance therapy but are not approved for use because of lack of survival benefit [72]. Similarly, there is a modest benefit in PFS without improvement in OS in recurrent OC after treatment with trebananib (anti-angiopoietin 1/2) [72].

The addition of bevacizumab to chemotherapy showed improved PFS in platinum-sensitive primary and recurrent OC [72]. In ICON7, the addition of bevacizumab to primary chemotherapy resulted in a modest improvement of PFS from 17.3 to 19 months in all patients and 10.5 to 15.9 months in high-risk patients with suboptimal debulking or stage IV disease. Latest ICON7 results (presented at the 2013 European Society of Gynaecologic Oncology meeting) showed no OS improvement in the entire cohort, but the high-risk subgroup had a significant prolongation of OS from 30.3 to 39.7 months with bevacizumab. Gourley showed that unsupervised clustering of 265 HGSOCs resulted in identification of two pro-angiogenic subgroups and one immune molecular subgroup. When a 63-gene signature biomarker developed to distinguish between these subgroups was applied to two different datasets of HGSOC, the immune subgroup had a significantly better PFS and OS than the angiogenic subgroup. More importantly, this biomarker was able to distinguish bevacizumab responders from non-responders in the ICON7 trial [73]. Addition of such signature biomarkers to the classification of OCs can predict response to anti-angiogenic therapy, thereby limiting treatment and toxicities.

D. Conclusions

Several meta-analyses evaluating biomarkers and their prognostic roles in OCs have been published over the last few years [9]. By using high-throughput genomic and proteomic analyses, studies are reporting unsupervised clustering of a large number of OCs into molecularly distinct subtypes that are associated with treatment response and clinical outcomes.

III. Cervical carcinoma

Current research efforts aim to discover molecular markers that will enhance or replace papanicolaou and human-papilloma virus (HPV) testing in screening and prevention of invasive disease. Diagnosis of pre-invasive disease has obvious benefits but here we focus on molecular prognostic markers of invasive disease. Recurrence and benefit from adjuvant radiation in stage IB patients depends on pathological findings of LVSI, cervical stromal involvement and tumor size [78]. Other post-surgical findings including lymph node involvement, and vaginal or parametrial margins are important in evaluating the risk of recurrence and the need for adjuvant therapy after radical surgery [79]. Numerous molecular markers have been investigated for ability to predict treatment response and clinical outcomes in early stage cervical cancer [80]. Despite positive association with clinical outcome, these biomarkers are not being used because of the lack of consistent studies with large sample sizes and prospective data.

A. Prognostic markers and targeted therapies

Angiogenesis markers

VEGF: High intra-tumor VEGF expression was related to poor PFS and OS in a series of 135 women with stage IB and IIA cervical cancer [81]. Another study including 173 cervical cancer (CC) patients treated with post-surgical radiotherapy because of lymph node or parametria or vaginal disease did not show a significant association between VEGF immunostaining and survival [82]. Thus VEGF may be a predictor of lymph node

involvement, and evaluation of this biomarker may be important in deciding treatment options when lymph node status is unknown.

HIF-1 α : Hypoxia-inducible factor 1 α is the active subunit of HIF-1 and plays an important role in oxygen homeostasis and adaptation of tumor cells to grow in unfavorable environments. Strong HIF-1 α expression was noted in 22% of stage IB CCs and was associated with poor PFS and OS [83].

Targeted therapies: The addition of bevacizumab to chemotherapy in advanced stage patients improved OS from 13.3 to 17 months [84].

HPV detection—In a cohort of 183 cervical squamous cell (SCC) and adenocarcinomas HPV was detected by PCR in 43% of tumors. Patients with HPV+ tumors were more likely to have complete response and disease remission than those with HPV-negative tumors [85]. A multivariate analysis adjusting for FIGO stage and histology showed HPV-negative status was associated with increased risk of recurrence and death [86]. The data suggests that HPV-positive CCs have a better prognosis when compared to HPV-negative CCs. HPV status is easy to determine by PCR and should be incorporated in routine assessments that guide treatment strategies.

B. Conclusions

Several other biomarkers have shown potential in predicting prognosis and response to treatment but results are from small studies. These include cell cycle regulators (p16, p21, p27, cyclin A/D/E), receptor tyrosine kinases (EGFR, HER2), metastatic or stem cell markers (CD44, Cathepsin D) and apoptotic markers (p53, Bcl-2, Bax). Overexpression of cyclooxygenase-2 (COX-2) and activation of PI3K/mTOR pathway has been hypothesized to inhibit apoptosis and promote carcinogenesis and angiogenesis [80]. Since COX-2 and PI3K inhibitors are already commercially available, their association needs to be studied in larger cohorts. Proliferation markers including p16, Ki-67 and p53 are associated with advanced stage and metastatic disease but they are not proven independent predictors of clinical outcomes and are not targetable for treatment [80]. The identification of targetable biomarkers is required to improve the poor prognosis of patients with advanced disease.

IV. Vulvar Carcinoma

Investigating the prognostic potential of biomarkers in vulvar cancer (VC) is problematic because most case series are small. For example, strong VEGF expression is associated with poor OS in a univariate analysis of 25 patients [87]. As in CC, p16, p21, VEGF, CD44, EGFR, and HER2 correlate to clinical outcomes in small studies [88]. In contrast, studies report conflicting evidence about the association between HPV and prognosis. Studies with larger cohorts and adjustment for known pathologic prognostic factors need to be performed.

A. Prognosis markers and targeted therapies

Matrix metalloproteinase 2—The enzyme MMP-2 degrades type IV collagen in the extracellular matrix, thereby disrupting the major structural components of the epithelial basement membrane. MMP-2 is present in 52% of VCs and immunoresponse is higher in

invasive carcinomas than in its precursors suggestion a role in carcinogenesis and invasion [89]. In a case series of 75 invasive vulvar cancers, MMP-2 expression was associated with better survival after adjusting for tumor size, depth of invasion, and patient age [90]. Specifically, more than 50% of MMP-2 expression correlated to a significant reduction in 5-year OS from 72.3% to 40%.

Targeted therapies: Nafamostat mesilate, a synthetic proteinase inhibitor decreased proliferation of SCC cell lines of the vulva and head and neck [91]. Targeting MMP-2 does not lead to cell death, but decreased MMP-2 expression may mimic a state of improved survival.

B. Conclusions

Although numerous molecular markers have been suggested to play a role vulvar carcinogenesis and prediction of clinical outcome, none are currently being used in clinical practice. Also, pre-clinical studies evaluating targeted therapies need to be performed.

In Summary

Although no gynecologic cancers are universally classified with molecular markers, this may be a staging strategy in the near future. With current genomic and proteomic platforms, we can generate high-throughput data leading to classification and analysis of cancer subtypes. Years of research have revealed that all gynecologic cancers have several subtypes and that genetic and molecular aberrations are responsible for heterogeneity in these diseases. Therefore, determining which genes and pathways are altered has clear potential to improve subtype classification. Molecular markers also show promise in predicting response to therapy and long-term clinical outcomes. Acquiring biomarker information in addition to cancer type and stage will allow us to personalize cancer treatments and surveillance after therapy.

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Practice points

- Gynecologic cancer stage is determined by physical extent of disease and metastasis as determined by physical exam and post-surgical pathologic findings.
- There is no universally accepted molecular classification strategy in staging for gynecologic cancers.
- Molecular markers associated with response to targeted therapies and clinical outcomes have been identified.
- With the advent of new sequencing technology, we can generate high throughput data to classify tumors on genomic and metabolomics platforms.
- New technologies should be employed to categorize cancers more efficiently and precisely in order to accurately predict response to treatment strategies and clinical outcome.

Research agenda

- Important prognostic factors currently used to predict outcome and guide choices for adjuvant therapy
- Molecular biomarkers associated with tumor heterogeneity
- Molecular biomarkers associated with clinical outcome
- The development and evaluation of targeted therapies
- The role of molecular markers in staging and treatment of gynecologic cancers

Highlights

Background

- There is no universally accepted molecular staging system for gynecologic cancers.

Role of molecular markers

- Different genetic alterations give rise to heterogeneity of gynecologic cancers.
- Biomarkers are associated with response to therapy and clinical outcomes.
- Novel drug therapies targeting molecular aberrations are being evaluated in clinical trials.

Conclusion and Future Perspective

- Genomic and proteomic characterization of gynecologic cancers is feasible and should be performed for accurate and comprehensive staging.