Clinical implications of using molecular diagnostics for ovarian cancers

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In the era of morphologic diagnostics, any epithelial tumor on or involving the ovaries was presumed to come from and be strictly of ovarian origin, apart from the rare but clearly metastatic tumors. Thus, many women who might have had small fallopian tube primary cancers that rapidly extended on to or into the ovary were deemed to have ovarian cancer. Now, as we begin to better understand that there are different types of cancers of nonuterine Muellarian origin, we expand upon the morphologic to add the molecular characteristics. Morphomolecular characteristics are being applied to drive clinical advances including development and optimization of predictive and prognostic biomarkers, redefinition of historical controls, and consideration of novel clinical trial designs. Ovarian cancer, not a common cancer to start with, is now subdivided into types, making ever smaller clinical cohorts. The first studies evaluating tubo-ovarian Muellarian cancers of morphomolecular types have begun. Deleterious mutations in BRCA1 or 2 have been validated as the first new predictive and prognostic biomarker of the high-grade serous ovarian cancer type and polyADPribose polymerase inhibitors, the first targeted agents for this morphomolecular entity. Similar progress is developing in other tubo-ovarian cancer types. This new knowledge is driving the building of a structure–function-type relationship that is generating novel clinically applicable hypotheses for testing.

**Key words:** morphological, biomarker, high-grade serous ovarian cancer, molecular, therapeutics

### is there a clinical need for recategorization of tubo-ovarian cancers?

For the longest time, we have called any epithelial malignancy emanating from the ovaries or tubes, or obscuring the ovaries and tubes by overgrowth, as being of ovarian origin, epithelial ovarian cancer (EOC). Progressive molecular genetics and careful pathologic and immunohistochemical examination, coupled with the drive to define the cell of origin, has uncovered trends that when applied analytically, initially segregated high-grade serous ovarian cancers (HGSOC) from all others [1–3], then allowed further characterization of the elements of type I cancers [4, 5]. We now have categories or types of tubo-ovarian cancers that make molecular and clinical sense [5]. These designations and studies have led to understanding the sources of the cancers, now shaping our thinking on early detection and prevention strategies, and focusing our therapeutic strategies. Thus, the implications of using molecular descriptors in characterizing nonuterine Muellarian tubo-ovarian cancers are broad.

Prospective application of typing of ovarian cancers will result in more homogeneous populations, albeit smaller populations, creating new challenges, as well as opportunities (Table 1). Will the prior decades of clinical advances be moot when we consider the new EOC categories when reviewing existing literature? GOG-111, the study that put paclitaxel into the international standard of care, had serous carcinoma in 64% versus 76% of cases, and the presence of and balance in low- and high-grade serous cancers is unknown [6]. Similarly, GOG-175 a study of early-stage patients receiving three cycles of carboplatin and paclitaxel and in one arm weekly paclitaxel maintenance was balanced for serous, endometrioid, mucinous, and clear-cell cancers [7]. Presently, there is controversy as to whether or not paclitaxel is a reasonable agent for any mucinous or clear-cell EOC, thus raising the question for over a third of the treated patients. The recategorization makes scientific and clinical sense. The key will be to see if we can improve care for women with the different types of EOC through this new dichotomization.

### is there a readily applicable diagnostic differentiation?

Overlaying the molecular findings over the morphological categories reinforces the distinctiveness of many of the categories and also, in some cases, underscored the probable similar underlying source (Table 2). Morphologic differentiation has been used for several decades and now coupled with a selected cassette of immunohistochemical markers, has been shown to discriminate the different types of EOC reliably and reproducibly. Kobel and coworkers described the use of HNF1β, MUC5, p16/p53, vimentin, and WT1 to clearly...
Quality of life (physical, emotional, financial) have been validated as an important biomarker predictive of clinical outcomes.

Optimal use of targeted agents (sequence/combination/timing) is more difficult.

'Historical control' outcomes will need to be redefined.

Populations will become smaller making practice changing trials (classical randomized phase III) more difficult.

Table 1. Clinical challenges and opportunities of application of morphomolecular characterization of nonuterine Muellerian cancers

<table>
<thead>
<tr>
<th>Challenges</th>
<th>Opportunities</th>
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<tr>
<td>Reproducible and reliable ovarian cancer-type identification</td>
<td>• Homogeneous patient populations</td>
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<tr>
<td>Populations will become smaller making practice changing trials (classical randomized phase III) more difficult</td>
<td>• More accurate prediction and prognostication</td>
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<td>Optimal use of targeted agents (sequence/combination/timing)</td>
<td>• Greater potential for development or reliable biomarkers</td>
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<td>Quality of life (physical, emotional, financial)</td>
<td>• Develop more reliable outcome descriptors</td>
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<td></td>
<td>• Collaborative/international programs</td>
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<td>• Targeted (or individualized) therapy</td>
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<td>• Avoiding and/or minimizing toxicity and costs related to unnecessary treatment</td>
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Separate high- and low-grade serous, clear-cell, endometrioid, and mucinous EOC [5, 8, 9]. Subsequent application of molecular analyses within a type of EOC may drive therapeutic decision-making. Deleterious germline BRCA1/2 mutations (BRCA1/2mut+) both define a subset of HGSOC, and also have therapeutic implications. Studies show that women with BRCA1/2mut+-associated ovarian cancer live longer and have better responses to platinum-based therapies [10, 11]. Mutations in ARID1a are found selectively in clear-cell and low-grade endometrioid EOC [12–15]. These mutations also have been seen in adjacent endometriotic implants, allowing for consensus that endometriosis is the (a) site of origin for these cancers. Thus, the addition of molecular and protein biomarker studies has led to cleaner and more consistent diagnosis.

**will application of morphomolecular categorization of EOC lead to more rapid diagnostic and predictive biomarker development?**

Predictive biomarkers discriminate for response to intervention and can do so among and between populations [16, 17]. Prognostic biomarkers are those that describe outcome, such as progression-free and overall survival. They may or may not have predictive power. The most reliable and robust biomarkers used in oncology practice today emanate from genetic characterization studies. For example, amplification of HER2 both defines a subset of breast and other cancers, and it has been validated as an important biomarker predictive of clinical benefit from the multitude of anti-HER2 therapies (trastuzumab, lapatinib, pertuzumab, and others) [18–20]. HER2 amplification has been seen in some cases of mucinous EOC; reports of therapeutic benefit from trastuzumab have validated it as a predictive biomarker where present [21, 22].

The most successful predictive biomarkers in oncology in general are gain-of-function mutations in oncogenes, such as activating KRAS, V600E-BRAF, and EGFR mutations. Gain-of-function or activating mutations tend to occur in reproducible places and often in relatively focused areas, as is well demonstrated for EGFR in lung cancer [23]. KRAS- and BRAF-activating mutations occur in over half of the low-grade serous EOCs [4, 24–27]. These low-grade cancers have been described as having a low chemosensitivity, where morphology is a negative predictive factor, based on retrospective studies, leading to consideration of these genetic gain-of-function changes as potential predictive biomarkers [28, 29]. GOG#239 examined the role of selmetinib in 52 low-grade serous cancers, on the hypothesis that ERK activation, downstream of both KRAS and BRAF, was a key event in these cancers [30].

Mutational analysis was reported for 34 patients of whom any RAS mutation was seen in 62% and BRAF mutation in 6%; contrary to the 68% mutation frequency was the 21% response rate in mutation carriers. Grisham and coworkers described a cohort of low malignant potential and low-grade serous patients in whom mutation was a positive prognostic factor for treatment-free overall survival rather than a poor outcome biomarker [25, 31]. Harmonizing these two findings will be important especially as the international community moves forward into another trial of MEK inhibition in this cohort.

Mutations in tumor suppressor genes are loss-of-function mutations; even the p53 mutation that results in high protein production is a loss-of-function mutation in that it removes the normal regulatory behavior of p53. Many genes required for normal cellular homeostasis, such as those in homologous recombination (HR) double-stranded DNA repair, such as BRCA1 and 2, have loss-of-function mutations. In fact, these are the most common genetic events seen in HGSOC. We have long recognized the subset of familial EOC as high risk for development of EOC, having high-grade serous histology, and having uniquely persistently platinum-sensitive disease that has led to improved overall survival. Thus, being BRCA1/2mut+ can serve as a cancer control biomarker to highlight patients for screening, a predictive biomarker for platinum sensitivity, and as a biomarker of good prognosis [10, 11, 32]. The key functions of BRCA1 and 2 defined to date are to maintain successful double-stranded DNA damage repair via the HR repair pathway [33]. Both proteins are needed for this high fidelity repair system to work; more recent results have shown germline mutations in several other genes in the HR pathway although the low-frequency and rare families precludes full characterization [34]. Nongermline loss of BRCA1/2 expression or function has not fully aligned with the BRCA1/2mut+ phenotype, such that BRCA-like cancers may not enjoy all the same benefits [32, 35].

The understanding of the role of BRCA1/2 in EOC has led to identification of the HR pathway genes as putative biomarkers of high-grade EOC and potentially as predictive biomarkers. TCGA and other molecular studies have shown that approximately half of HGSOC have some abnormality in HR [34, 36–38]. These abnormalities segregate in high-grade serous and endometrioid EOC, with recurrent platinum sensitivity, and suggestion of improved prognosis. Further validation of other HR genes/proteins as predictive biomarkers in EOC is needed.
Bowtell and coworkers and others have identified cyclin E amplification in a subset of HGSOC without BRCA1/2mut+ [39–41]. Prognostic importance was demonstrated for progression-free and overall survival. Modulation of cyclin E in vitro credentials it as a biomarker of outcome and suggests its value as a potential therapeutic target. Numerous cyclin-dependent kinase inhibitors are in development and could be an important direction for testing for women with cyclin E amplification.

**can we use morphomolecular characteristics for therapeutic targeting or as therapeutic targets?**

Therapeutic targeting implies application of a biomarker as a selection criterion but does not require the biomarker to be the therapeutic target; whereas, an entity can be both a biomarker and the target against which a therapeutic is focused. BRCA1/2mut+ characterize a proportion of the HGSOC type of EOC and function as both predictive and prognostic biomarkers. To date, tumor suppressor genes have not been successfully directly therapeutically targeted. However, involved pathways have been and the HR/BRCA1/2mut+ is a canonical example, credentialing BRCA1/2mut+ as an integral predictive biomarker. The HR pathway is complex, has many steps, and involves a multitude of gene products, many of which have been shown themselves to be mutated either at the germline or somatically in HGSOC [33, 34, 42, 43]. HR is the dominant pathway and when dysfunctional, such as in BRCA1/2mut+, the base excision repair single-strand DNA break pathway (BER) and/or the poor fidelity double-stranded DNA repair nonhomologous end-joining pathway (NHEJ) are activated [33, 44, 45]. The rate-limiting step in BER is activation of polyADP ribose polymerase (PARP), and its subsequent PARylation decoration of the single-strand break point on the DNA [46]. This serves as a flag to subsequent proteins that repair is needed. In the absence of HR, BER is activated, requiring PARP function [45, 47]. This is the tenet of the new category of PARP inhibitors and also explains their higher activity in BRCA1/2mut+ patients. PARP may also function to keep NHEJ in check, such that when it is inhibited, the poor fidelity NHEJ program is activated and even more errors of repair occur, ultimately triggering apoptosis [44]. Herein, the molecular characteristics of BRCA1/2mut+ indirectly define both whom to target and what elements may be successfully targeted.

Enter BRCA-like behavior, molecular and clinical characteristics similar to those seen in BRCA1/2mut+ patients but in the absence of germline mutations [38]. Many mechanisms reducing BRCA1/2 function and resulting in BRCA-like behavior have been identified. As seen in the TCGA report [36], at least half of HGSOCs may have potential HR dysfunction (HRD). Does the morphomolecular characterization accurately predict response to the PARP inhibitor family? The simple answer is both yes and no. There are no data to date in non-BRCA1/2mut+ EOCs of nonhigh-grade serous histology to help answer this directly. BRCA1/2mut+ breast cancer is nearly 75% of the triple negative (TNBC; ER/PR/HER2 negative) category, and we and others have observed

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poor PARP inhibitor response frequency and duration in non-BRCA1/2mut+ TNBC. This can be addressed at a different level in HGSOC. There are at least three major subtypes of HGSOC all of which still have p53 mutation/dysfunction: BRCA1/2mut+ associated, BRCA-like (HRD based on mutation or amplification in other genes, BRCA1/2 methylation, and other changes), and non-HRD (CCNE1 amplified, for example). There is a clear dichotomy of activity to PARPi within the groups characterized by HRD-related biomarkers. Gelmon and coworkers presented activity of the PARPi olaparib in both cohorts, but with a shorter duration and approximately half as much activity in the HRD+ but BRCA1/2mut+-negative group [35]. Lee and coworkers presented a similar relationship between HRD BRCA1/2mut+-positive and -negative HGSOC cohorts in their study of olaparib with carboplatin [48]. Notably, they found activity of the combination even in platinum-resistant patients.

Clear-cell cancer is characterized in part by ARID1a mutations with associated loss of BAF250a [12, 14, 15]. It is not yet understood how or why this mutation segregates in clear-cell and low-grade endometrioid EOC, thus applying these findings as predictive markers or therapeutic targets is premature. Clear-cell cancer of the kidney is recognized to have a hypoxic drive due to dysfunctional regulation of the VEGF inducer, HIF1α [49]. Work by Angelesco and Bowtell demonstrated that ovarian clear-cell cancers also have a hypoxic drive [22]. They demonstrate a gain-of-function behavior via upregulation of HIF2α and HNF1α in an IL6→SRC/LYN family kinase→STAT3 sensitive pathway. Clear-cells characterized morphomolecularly and confirmed by HNF1α expression may have increased sensitivity to SRC family kinase inhibitors such as dasatinib or by inhibition of the IL6 pathway.

These considerations may be integrated and applied to treatments. EOC is a relatively rare tumor worldwide and use of morphomolecular typing will further narrow power to address clinical questions. Novel trial designs will be needed to enrich patients within a tumor type and to rapidly evaluate potential agents in a world of increasingly greater numbers of drugs within a category and ever more potential entities to target. There may be many ways to synthesize use of the new morphomolecular characterization of EOC into clinical trial designs (Table 2). One such approach is shown in Figure 1.

the time has come

The genetic revolution has changed categorization and clinical intervention in many solid tumors, improving outcome for patients with those cancers. We have made major progress in ovarian cancers in the last few years, understanding that, once again, ovarian cancer does not follow in the footsteps of other solid tumors [31] but forges its own way. We recognize that high-grade serous cancers have marked genomic instability rather than sets of driving mutations, that KRAS and BRAF mutations may not portend poor outcome in the low-grade serous cancers, and that they may not predict susceptibility to downstream pathway targeted agents. The mutations in ARID1a have taught us about the link between clear-cell and low-grade endometrioid ‘ovarian’ cancers and endometriosis, but have yet to give us therapeutic direction. Nonetheless, the progress is striking and the use of morphomolecular criteria in nonuterine Muellerian tubo-ovarian cancers is here to stay.

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disclosure

The authors have declared no conflicts of interest.

references


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