Review

Next-Generation Sequencing: Role in Gynecologic Cancers

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Abstract

Next-generation sequencing (NGS) has risen to the forefront of tumor analysis and has enabled unprecedented advances in the molecular profiling of solid tumors. Through massively parallel sequencing, previously unrecognized genomic alterations have been unveiled in many malignancies, including gynecologic cancers, thus expanding the potential repertoire for the use of targeted therapies. NGS has expanded the understanding of the genomic foundation of gynecologic malignancies and has allowed identification of germline and somatic mutations associated with cancer development, enabled tumor reclassification, and helped determine mechanisms of treatment resistance. NGS has also facilitated rationale therapeutic strategies based on actionable molecular aberrations. However, issues remain regarding cost and clinical utility. This review covers NGS analysis of and its impact thus far on gynecologic cancers, specifically ovarian, endometrial, cervical, and vulvar cancers.

J Natl Compr Canc Netw 2016;14(9):1165–1173

Malignancies of the female genital tract are a consequence of genomic alterations influenced by heritable and acquired mutations, transcriptional deviations, and epigenetic factors. Understanding the genomic and molecular foundation of gynecologic malignancies is essential for the discovery of novel biomarkers for screening and prevention, molecular understanding, and development of more effective treatment strategies. Traditionally, genetic analysis of solid tumors involved analyzing small, single portions of DNA, known as *Sanger sequencing*.[1] Although Sanger sequencing has proven validation for determining the sequence of a known particular gene, its use in sequencing multiple genes concurrently is limited, time-consuming, and costly. Next-generation sequencing (NGS) allows thousands to millions of DNA fragments to be sequenced simultaneously in a single assay, thereby expanding the amount of genetic information obtained and decreasing both time to perform the assay and cost.[2,3] For certain malignancies, such as lung cancer, the clinical implications of NGS have been profound and have led to rationale-targeted therapy development[4]; in gynecologic cancers, the use of NGS has yielded important genomic understanding of cancers and identification of high-risk inherited genes, but its use in treatment remains limited.[5,6] This review discusses the impact of NGS on the understanding of molecular pathogenesis, cancer screening and prevention, diagnosis, and therapeutic strategies for gynecologic malignancies.

NGS and Molecular Analysis of Gynecologic Cancers

Ovarian Cancer

One of the most important and influential uses of NGS occurred with The Cancer Genome Atlas (TCGA) project, a multinational collaboration to systematically characterize the genomic and epigenomic signatures of human

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Submitted March 22, 2016; accepted for publication July 25, 2016.

The authors have disclosed that they have no financial interests, arrangements, affiliations, or commercial interests with the manufacturers of any products discussed in this article or their competitors. Dr. Matulonis has disclosed that she is a scientific advisor for Genentech, Inc./Roche, ImmunoGen, Merck & Co, Inc., and Pfizer Inc.; receives consulting fees from AstraZeneca Pharmaceuticals LP; and is an unpaid consultant for AstraZeneca Pharmaceuticals LP.

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cancers, including gynecologic cancers.[7] In 2011, the TCGA Research Network published its results from the genomic evaluation of 489 high-grade serous ovarian carcinomas (HGSOCs) before treatment with systemic chemotherapy.[7] NGS provided whole-exome DNA sequences for 316 of the HGSOC specimens. Among the many identified alterations were 168 epigenetically silenced genes due to promoter methylation changes; 9 genes with significant recurrent mutations, including *TP53*, *BRCA1*, *BRCA2*, *NF1*, *CSMD3*, *CDK12*, *FAT3*, *GABRA6*, and *RB1*, from a constellation of nearly 19,000 somatic mutations; and 113 DNA copy number mutations present in HGSOCs.[7]

The TCGA data have been instrumental in demonstrating the presence of homologous recombination deficiency (HRD) in approximately 50% of all HGSOCs, thus having therapeutic implications (Table 1), in addition to influencing the development of rational clinical trial design for HGSOCs.[7] HRD is mostly conferred through mutations in either *BRCA1* or *BRCA2*, but many other DNA repair genes also play a role conferring the HRD genotype.[7,8] Mutations in the *TP53* gene have been found in nearly all HGSOC tumors.[7,9] *TP53* mutations typically occur in DNA binding domains, but can also occur in non–DNA binding domains and involve in-frame and frameshift insertions and deletions as well as missense mutations or nonsense mutations.[7,9,10] The TCGA data showed DNA binding domain missense mutation in 58.5% of cancers.[7,9] Seagle et al,[10] using the TCGA HGSOC data, demonstrated that the location of the mutation in the p53 structure influenced outcome; cancers with aberrations in the DNA major groove residues or zinc ion coordinating residues had the best outcome, whereas sheet-loop-helix stabilizer changes had overall poorer outcome. In ovarian cancers that do not harbor *TP53* mutations, p53 dysfunction has been demonstrated with *MDM2* or *MDM4* copy number gain.[9] The TCGA has also noted aberrations in other pathways besides DNA repair in HGSOCs, such as PI3 kinase/RAS, NOTCH, and FOXM1 transcription factor network.[7] The integrated analysis of HGSOCs has highlighted the genomic complexity of this cancer and has provided critical genomic information that has prognostic and therapeutic significance, as discussed later in this review (Table 1).

Most patients with newly diagnosed advanced HGSOC experience an excellent response to platinum-based chemotherapy, but when it recurs, the cancer becomes increasingly resistant to platinum, which is the most active agent against this cancer. In efforts to explore the molecular properties influencing platinum-resistant and -refractory disease, Patch et al[11] used whole-exome sequencing to examine HGSOC and demonstrated the broad range of molecular changes that accompany the emergence of platinum resistance, which included gene breakage and subsequent inactivation of several tumor suppressor genes, such as *RB1*, *NF1*, *RAD51B*, and

Table 1. Examples of Clinically Impactful Findings From NGS in Gynecologic Cancers

Genomic Finding	Clinical Observation or Action
Germline *BRCA1* or *BRCA2* mutation	Risk-reducing surgeries for ovarian and breast cancer[58,59]
Germline mutations in *BRIP1*, *RAD51C*, *RAD51D*, *PALB2*, *BARD1*, *MSH2*, *MLH1*, *PMS2*, *MSH6*	Entire group of genes: increased risk of ovarian cancer[27–30] *MSH2*, *MLH1*, *PMS2*, *MSH6*: Increased risk of ovarian, endometrial, colorectal cancers[28]
Somatic *BRCA1* or *BRCA2* mutation on NGS of ovarian cancer	Improved overall survival compared with *BRCA* wild-type cancers and increased susceptibility to poly(ADP-ribose) polymerase inhibitors and platinum agents[7,48,53–55] Possible increased sensitivity to immunotherapy agents[64]
Cycling E and MDR1 amplification Inactivation of RB1, NF1, RAD51B, PTEN *BRCA* reversion mutations in HGSOC	Enhanced resistance to platinum chemotherapy[11]
POLE mutations in endometrial cancer	Possible increased sensitivity to checkpoint blockade inhibitors[65]
Structurally grouped *TP53* mutations	Missense mutation at the R248 location of *TP53* increases taxane and platinum resistance for HGSOC[10]

Abbreviations: HGSOC, high-grade serous ovarian carcinoma; NGS, next-generation sequencing; POLE, polymerase ε.

PTEN, which contributed to acquired platinum resistance. Also identified by this group was amplification of coding proteins such as *CCNE1*, which was associated with primary platinum resistance. Other events found implicated in acquired platinum resistance included *BRCA1* and *BRCA2* reversion mutations and loss of *BRCA1* promoter methylation.[11] In addition, Patch et al[11] showed that in 8% of platinum-resistant cancers, there was upregulation of multidrug-resistant protein 1 (MDR1) protein as a result of promoter fusion and translocation involving the 5′ region of the *ABCB1* gene, which encodes MDR1; the MDR1 protein is a chemotherapy efflux pump for agents such as paclitaxel and doxorubicin. Several of the alterations found by Patch et al represent potentially therapeutic targets, such as *CCNE1* amplification or MDR1 upregulation.

Other less common ovarian cancer subtypes have undergone NGS, including low-grade serous (LGSOC), mucinous, clear cell, and small cell cancers. The mitogen-activated protein kinase (MAPK) pathway has been found to be activated in up to 80% of LGSOC with infrequent *TP53* mutations.[12] Through whole-exome sequencing of LGSOC, Hunter et al[13] identified 2 previously unidentified protein coding genes in the disease: eukaryotic translation initiation factor 1A, X-linked (EIF1AX) and ubiquitin-specific peptidase 9X (USP9X), both of which are associated with mTOR regulation. EIF1AX and USP9X had substantially higher expression in LGSOC compared with serous borderline tumors, which may implicate these genes in the pathogenesis of LGSOC and may suggest a role for mTOR inhibitors to improve treatment response in the relatively chemoresistant LGSOCs. Similarly, using whole transcriptome analysis, researchers have identified mutations of tumor suppressor gene *ARID1A* to play a key role in the pathogenesis of many clear cell cancers and ovarian endometrioid carcinomas, but not in HGSOCs.[14,15] Others have also identified HNF1B overexpression in clear cell cancers.[15] Pennington and Swisher[16] demonstrated that HRD gene germline or somatic mutations were found in other histologies besides HGSOC, including clear cell, endometrioid, and carcinosarcoma having potential therapeutic and prognostic implications. Mucinous cancers, which are typically chemotherapy-resistant, harbor *KRAS* mutations, possibly explaining this chemotherapy insensitivity.[17,18] Ryland et al[18] recently performed exome sequencing of mucinous tumors that included benign, borderline, and carcinoma histologies; they showed mutations in *KRAS*, *BRAF*, and *CDKN2A*, and recurrent mutations in *RNF43*, *ELF3*, *GNAS*, *ERBB3*, and *KLF5*, in addition to *TP53* mutations. Small cell carcinomas of the ovary, hypercalcemic type, which are very aggressive cancers occurring mostly in younger women, have been found to have a *SMARCA4* somatic or germline mutation.[19,20]

Endometrial Cancer

In addition to ovarian cancer, the TCGA Research Network has also evaluated 373 endometrial carcinomas using NGS methods, which has challenged the traditional endometrial cancer classification system of type 1 (estrogen-dependent) and type 2 (aggressive non–estrogen-dependent), with identification of 4 classes of disease: polymerase ε (POLE) ultramutated, microsatellite instability hypermutated, copy-number low, and copy-number high consisting of mainly serous cancers.[21] Uterine serous carcinomas displayed genomic similarities with HGSOC and triple-negative breast cancer with frequent *TP53* mutations, high copy number variation, and low mutational burden.[21] Endometrioid cancers demonstrated few copy number alterations or *TP53* mutations, but instead had mutations in *PTEN*, *CTNNB1*, *PIK3CA*, *ARID1A*, *ARID5B*, and *KRAS*.[21] The PI3K pathway is frequently abnormal in endometrial cancer and in fact, of all the cancers studied in TCGA, had the highest frequency of mutations in the PI3K/AKT pathway,[21] spurring many clinical trials of PI3K inhibitors, but these agents have exhibited disappointing single-agent response rates. Endometrioid endometrial carcinomas have a 40% frequency of microsatellite instability and 7% POLE mutations, both leading to high mutation frequency and thus may predict higher responsiveness to immunotherapies (Table 1).[21] Fibroblast growth factor receptor (*FGFR*) has also been shown to be aberrant in 13% of all endometrial cancers, and Helsten et al[22] has identified FGFR, mostly *FGFR2* mutations, as being altered in endometrial cancers; this gene is altered in other gynecologic cancers and may represent a targetable mutation.[23]

Currently, histologic subtype and stage currently guide adjuvant treatment strategies in endometrial cancer, but the reclassification of endometrial cancer through the TCGA analysis could have future impli-

cations for management decisions and outcome prediction. Additionally, NGS technologies facilitated cross-tumor comparisons noting similar somatic copy number alterations, DNA methylation changes, and high *TP53* mutations with low *PTEN* mutations among uterine serous carcinomas, HGSOCs, and basal-like breast carcinomas.[21] These findings have future implications for cross-tumor treatment strategies targeting molecular abnormalities regardless of primary tumor site.

Cervical and Vulvar Cancers

The use of an NGS modality in analyzing cervical cancer using a mass spectrometry-based genotyping platform with mutation validation performed by chemistry using a multibase extension (homogeneous mass EXTEND [hME]; Sequenom, Inc., San Diego, CA) on native and nonamplified genomic DNA delineated the frequency of actionable mutations in cervical squamous cell carcinoma and adenocarcinoma.[24] Wright et al[24] showed that mutations in *PIK3CA*, *KRAS*, and *EGFR* were present in 31.3%, 8.8%, and 3.8% of cervical cancers, respectively, with *PIK3CA* mutations portending a worse prognosis, and *EGFR* mutations identified solely with squamous histology.

Another group using whole-exome, transcriptome, and genome sequencing of cervical cancers uncovered several previously unknown mutations in cervical squamous carcinomas and adenocarcinomas.[25] These genetic mutations involved *FBXW7*, *MAPK1* E322K substitutions, HLA-B inactivations, and *EP300* mutations as well as somatic mutations in *ELF3* and *CBFB* genes occurring in cervical adenocarcinomas, highlighting the role of epigenetic factors in cancer development.[25]

Vulvar cancer is a less commonly diagnosed gynecologic cancer, and pathogenesis of this cancer has been divided into a human papillomavirus (HPV)–dependent route and an HPV-independent pathway where lichen sclerosis and genetic mutations, such as *TP53*, are present.[26] HPV-associated vulvar cancers account for up to 40% of all cases, occur in younger women, and are associated with smoking; vulvar cancer not associated with HPV occurs in older women. The genetics of vulvar cancer are reviewed by Trietsch et al.[26]

NGS and Clinical Implications

Genetic Risk Assessment

During the past decade, NGS panels of genes associated with gynecologic malignancies have become important tools in counseling patients and families concerning cancer risk, risk-reduction planning, and available treatment options. Most notably for gynecologic cancers, these panels include genetic assessment for hereditary breast and ovarian cancer mutations (ie, *BRCA1* and *BRCA2*) and Lynch syndrome–related genes (ie, *MLH1*, *MSH2*, *MSH6*, *PMS2*) with associated risk of endometrial, ovarian, colorectal, and other carcinomas. Regarding targeted sequencing, Walsh et al[27] successfully used NGS to determine single-nucleotide substitutions, insertion and deletion mutations, and duplications and deletions without any false-positives for genetic changes, including nonsense and frame-shift mutations for 21 tumor suppressor genes associated with breast and ovarian cancer tested. Of the 360 patients tested, Walsh et al[27] determined that 24% of these patients harbored germline loss-of-function mutations: 18% in *BRCA1* or *BRCA2* and 6% in *BARD1*, *BRIP1*, *CHEK2*, *MRE11A*, *MSH6*, *NBN*, *PALB2*, *RAD50*, *RAD51C*, or *TP53*. Of the women with inherited germline mutations, more than 30% of them had no documented family history of ovarian or breast cancer. Norquist et al[28] expanded this database, added cases from phase III studies from the Gynecologic Oncology Group, and sequenced germline DNA from 1,915 patients with ovarian cancer. Eleven genes were identified to cause hereditary ovarian cancer: *BRCA1*, *BRCA2*, *BRIP1*, *RAD51C*, *RAD51D*, *BARD1*, *PALB2*, *MSH2*, *MLH1*, *PMS2*, and MSH6; 18% of this cohort of patients carried a germline mutation increasing ovarian cancer risk. Germline mutations were not identified in mucinous histologies, although only 16 cases were analyzed, and Lynch syndrome mismatch repair genes (*MSH2*, *MSH6*, *PMS2*, *MLH1*) were rare, present in only 0.4% of patients.[28] Other groups have also demonstrated the importance of germline mutations in *RAD51C*, *RAD51D*, and *BRIP1* in inherited risk of ovarian cancer.[29,30] Targeted NGS may facilitate broader evaluation of heritable mutations beyond *BRCA1* and *BRCA2*, and the studies mentioned highlight the importance of genetic testing in all ovarian cancers. In fact, the NCCN Clinical Practice Guidelines in Oncology for Genetic/Familial

High-Risk Assessment: Breast and Ovarian now recommend genetic testing for all women with a diagnosis of ovarian cancer, regardless of age, family history, or histologic subtype.[31] Clinicians and patients have access to a growing number of companies with multiple available panels, with a varied number of gynecologic cancer–associated genes sequenced, several of which use NGS.[32–35]

Although NGS panels for genetic risk assessment can provide an accurate, timely, and increasingly reasonably priced identification of numerous genes, one of the major concerns with multigene NGS is the ambiguity surrounding identification of genes with imprecise clinical relevance, and how these results influence clinical decision-making. A recent review examining the use of NGS in hereditary breast and gynecologic cancers found up to a 16% prevalence of deleterious non-*BRCA1* and non-*BRCA2* mutations, with detection of variants with unknown significance ranging from 15% to 88%[35]; the clinical significance of *BRCA* variants of uncertain significance is reviewed in Eccles et al.[36] Although the potential for a high prevalence of identifying genes with imprecise relationships to malignancy can hamper risk stratification, it is also important to note the ability of NGS panels to identify genomic variants with known gynecologic malignancy associations, thus broadening the diagnostic capacity of a single test. However, even with the identification of genes with better-defined cancer risk assessment, limited data are available examining the use of NGS techniques in cancer risk assessment.

Cervical cancer is unique among nonbreast gynecologic malignancies in having a recognized screening modality and well-established DNA biomarkers: high-risk HPV (HR-HPV) subtypes 16 and 18, accounting for most cervical cancers.[37,38] In April 2014, the FDA approved the cobas HPV test (a low-throughput test) in primary screening for cervical cancer for women ages 25 to 29 years. Although this test has proven validity,[39,40] it does not allow high-throughput genotyping of all HR-HPV types, which may be useful for evaluating a broader range of HPV subtypes for cervical cancer risk assessment.

Given the known value of HPV genotyping for cervical cancer risk stratification, and potentially increased use of primary HR-HPV testing, NGS may provide a valuable means to further delineate the clinical significance of identified DNA-based biomarkers and HPV integration. Mirabello et al[41] used NGS biochemical assays to determine DNA methylation status of HPV 16 infections from cervical cell specimens in women with HPV 16 DNA. Through determination of the methylation status of individual DNA molecules, researchers were able to differentiate transient HPV16 infections from HPV16 hypermethylated infections likely to represent true cervical cancer precursor infections.[41] Furthermore, several studies have supported the use of NGS as a sensitive and accurate means for HPV genotyping, highlighting that the enhanced analytics of NGS may improve risk stratification for patients with high-risk HPV subtypes.[42–46] With further study, this use of NGS for screening and diagnostics may provide a more robust, clinically relevant, and cost-effective means of determining HPV status.

NGS and Prognostic Information

Stage at diagnosis and recurrent disease continues to be one of the most important prognostic factors in gynecologic malignancies, stressing the necessity for sensitive and specific disease-related biomarkers for early detection, and treatment-related biomarkers for monitoring treatment efficacy. Based on the TCGA data and countless other studies, profound tumor heterogeneity exists by which various genes are mutated within the same malignancy or histologic subtype, or identical genes are altered in different malignancies.[7,12,21,24,25,47]

NGS has provided valuable information regarding prognosis in gynecologic malignancies. A notable example is the improved survival of patients with either germline or somatic *BRCA* mutations compared with patients with ovarian cancer with wild-type *BRCA* mutations or non-*BRCA* mutations (Table 1).[7,48] In addition, the presence of a *BRCA2* mutation appears to confer a better survival advantage than a *BRCA1* mutation.[48] In ovarian clear cell cancers, NGS has identified genes *ARID1A* (tumor-suppressor) and *PPP2R1A* (oncogene) as novel biomarkers in the disease[14,49]; loss of ARID1A protein expression in clear cell ovarian cancer as measured by immunohistochemistry has been shown to confer shorter progression-free survival and chemoresistance.[50]

The endometrial cancer TCGA demonstrated survival differences among the 4 identified molecular subgroups, with the ultramutated group having

the best survival and the serous cohort having the poorest overall survival.[21] Clinically, these subgroups are not used currently but may be in the future, with potential prognostic and clinical trial design implications. The presence of a *PIK3CA* mutation in cervical cancers portended a worse prognosis.[24]

NGS and Therapeutic Implications

Ovarian Cancer Subtypes and Identification of Therapeutic Strategies

Identification of HRD and Sensitivity to Platinum and Poly(ADP-Ribose) Polymerase Inhibitors: NGS has provided important information on the molecular composition of the various molecular subtypes of ovarian cancer. Currently, all patients with newly diagnosed high-risk epithelial ovarian carcinomas regardless of subgroup are treated with platinum-based regimens. However, with increasing data supporting differing clinical courses and response rates to standard chemotherapy, it is increasingly relevant to consider these subtype-specific genomic variations of epithelial ovarian cancers in the study of therapeutic strategies.[51,52] Although nearly 80% of patients with HGSOC respond to platinum-based chemotherapy, less than 27% of patients with ovarian clear cell carcinoma, and less than 5% of patients with LGSOC respond to such treatment[7,12,51]; these intrinsic histologic and molecularly based differences in response to platinum-based chemotherapy will need further study and require rational clinical trial design in order to make therapeutic advances.

The TCGA and others have demonstrated the presence of HRD genotype in approximately 50% of HGSOCs; this finding has implications for the use of platinum and agents called *poly(ADP-ribose) polymerase (PARP) inhibitors* (Table 1). PARP inhibitors such as olaparib have demonstrated efficacy in ovarian cancers that are more susceptible to therapies targeting HRD, such as platinum-sensitive HGSOCs.[53,54] A major breakthrough in targeted therapy for epithelial ovarian cancers occurred in December 2014 with the FDA approval of olaparib for the treatment of patients with recurrent ovarian cancer with a germline *BRCA* mutation previously treated with 3 or more lines of chemotherapy.[55,56] The use of olaparib in the study population demonstrated a 34% objective response rate with a median response duration of 7.9 months.[55] The European Medicines Agency also approved olaparib as a maintenance therapy for patients with a germline or somatic *BRCA* mutation based on the results of Study 19, which tested olaparib versus placebo in patients who have platinum-sensitive recurrent ovarian cancer that responds to platinum-based therapy.[53,54]

Low-Grade Serous Carcinoma and MAP Kinase Pathway Alterations: Low-grade serous carcinomas (LGSCs) have been demonstrated to have MAP kinase pathway abnormalities, and MEK inhibitor strategies have been attempted in this cancer.[12] A single-arm phase II study of selumetinib has shown promising activity in LGSCs, leading to several phase III strategies with mixed results thus far.[57] One such study that tested the MEK inhibitor MEK162 against physician's choice chemotherapy recently closed due to futility based on a planned interim analysis of progression-free survival (ClinicalTrials.gov identifier: NCT01849874), and another study is on clinical hold because of a trametinib shortage (ClinicalTrials.gov identifier: NCT02101788).

Identification of High-Risk Individuals for Inherited Ovarian and Endometrial Cancer

Identification of individuals who are at higher risk for developing ovarian and endometrial cancer has been a very significant development. With the identification of high-risk individuals, risk-reducing surgeries can be performed to prevent these cancers and are therefore now part of prevention guideline recommendations (Table 1).[58,59] Mutational location in high-risk genes can also have implications for risk and median age of onset of the cancer diagnosis; this has been demonstrated for the *BRCA1* or *BRCA2* genes, and knowledge of this information can thus help patients understand their own risk of cancer and guide the timing of risk-reducing surgery.[60]

Cost

Although the cost of NGS has recently decreased significantly, it depends on several factors, such as the setting where the testing is being performed (academic research vs clinical use) and the extent of sequencing.[61,62] Academic institutions performing NGS on cancers for research purposes will incur tissue collection, preparation, sequencing, and personnel costs.[63] Commercial testing may be covered by insurance for *BRCA1* and *BRCA2* analysis and

extended panel testing for patients with ovarian cancer. As additional genes are identified that are clinically actionable,[28] genes will be added to panel testing. For patients who had NGS performed previously on their cancer, financial coverage for these subsequent tests may be more uncertain.[61,62] Other NGS tests, such as somatic tumor profiling, may not be fully covered for patients with gynecologic cancer because of unknown or no known clinical benefit.[62] Tests such as FoundationOne for solid tumors, which examines more than 300 cancer-related somatic genes, costs $5,800, as listed on the Web site (foundationmedicine.com), and patients may incur some of the costs of these and other tests if insurance does not fully cover them, especially for gynecologic cancers. In addition, cost-effectiveness analyses will need to be performed on NGS tests for gynecologic and other cancers, such as those that have already been performed for *BRCA* analysis in high-risk populations and hereditary nonpolyposis colorectal cancer gene panels in colorectal cancer.[62,64,65]

Immunotherapy and NGS

Immune modulators in the treatment of gynecologic cancers are a growing area of interest, especially in the setting of tumor resistance to standard chemotherapies or viral-associated malignancies, such as cervical and vulvar cancers. NGS technologies have enabled massive analysis of antigen receptors and improved characterization of tumor-infiltrating T cells and receptors, which may have implications for targeted immunotherapies, and the role of tumor-infiltrating T cells in ovarian, endometrial, and cervical cancers is being explored.[66–68] Strickland et al[69] examined the immune differences of *BRCA*-mutated versus *BRCA* wild-type HGSOC and examined neoantigen load and found higher neoantigen load in *BRCA*-mutated cancers along with better outcomes associated with these cancers. In addition, *BRCA*-mutated cancers had higher amounts of infiltrating CD3 and CD8 tumor-infiltrating lymphocyte cells and higher expression of PD-1 and PD-L1 compared with *BRCA* wild-type HGSOC, suggesting that *BRCA*-mutated cancers may respond better to immunotherapy strategies compared with *BRCA* wild-type, HR-proficient cancers.[69] Howitt et al found that PD-L1 and PD-1 were frequently expressed in the immune cells of endometrial cancers with microsatellite instability and POLE mutations suggesting a role for anti–PD-1 immunotherapies in endometrial cancers; in fact, recent reports of the use of single-agent PD-1 inhibitors in these cancers appears promising.[71] NGS may prove to be a valuable tool for enhancing the characterization of immune cells and receptors in gynecologic malignancies to aid in the development and use of immunotherapy.

Discussion

NGS technologies have contributed to the understanding of the biologic processes influencing cancer development, progression, and response to treatment. Through the use of NGS, comprehensive molecular analysis of the histologic subtypes of gynecologic malignancies have revealed a wide spectrum of mutations and highlights subtype-specific molecular differences in the pathogenesis of disease.[5,7,21,24,25] Although there is still much to learn about the complex genetic, transcriptional, and epigenetic factors influencing gynecologic malignancies, NGS of tumor cells has provided the start toward addressing unanswered and important questions of cancer biology in gynecologic cancers.

Central to the utility and justification of NGS over traditional sequencing technologies is validation of the technique and effectiveness of applying acquired information to the clinical setting.[72] For example, in using NGS for HPV genotyping, Barzon et al[44] defined the benefits of NGS over low-throughput technologies as the ability to expand beyond HPV type identification, and distinguish the presence of multiple subtypes, variations, and potentially previously undiscovered types of high-risk HPV. In the case of endometrial and ov\arian cancers, NGS may aid in more accurate disease classification, which is beneficial for providing prognostic information and guiding therapy.[7,21] Through comprehensive NGS genomic analysis, we may identify a greater number of patients who are eligible for clinical trials, or identify novel variations for development of new drugs and additional clinical trials. From the available literature in gynecologic malignancies, NGS is likely to have its greatest clinical impact in discovery of new biomarkers for screening and early detection, detection of heritable mutations, monitoring treatment response or likelihood of response, and identification of new genomic alterations for targeted therapies. In addition, in the future, NGS technologies

may also be used to evaluate small amounts of DNA in ascites or blood where tumor cells are scant.[73] In gynecologic cancers, the use of circulating DNA is just beginning to be used clinically and in research[74]; this is potentially a very exciting and useful tool in gynecologic cancers.

Understandably, one area of concern with massively parallel sequencing is identification of genomic variations with unknown clinical significance, and how or if this information should influence clinical decision-making. Although NGS technologies have enabled confirmation of previously hypothesized ovarian cancer–associated homologous recombination genes, such as *BRIP1*, *RAD51C*, and *RAD51D*, the most appropriate way to guide patients without disease harboring these mutations remains unanswered at this time.[28] Additionally, how do we apply this information to treatment initiation or continuation in patients with disease? Although these questions are important to consider, comprehensive genomic characterization, whether novel or known, opens new areas for cancer research to improve understanding of disease processes. We must also consider the potential of NGS to recognize the presence of unexpected mutations with known targeted therapeutics, which may alter management, and identify molecular similarities and differences across different cancer histologies. This has been clearly demonstrated in cervical cancers with *EGFR* variants observed in squamous cell carcinomas but not adenocarcinomas, demonstrating the differences in molecular tumorigenesis.[24] With the growth of precision medicine, NGS technologies are likely to play a critical role in expanding our knowledge of genomic alterations and actionable mutations in gynecologic cancers. Although promising, additional studies are needed to validate NGS techniques, determine cost-effectiveness, and evaluate patient outcomes associated with NGS data used in panels for genetic risk assessment, biomarker detection, or targetable mutations.

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