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NCCN Guidelines Panel*:* Genetic/Familial High Risk Assessment: Breast and Ovarian

On behalf of Ambry Genetics, I respectfully request the NCCN (Genetic/Familial High Risk Assessment: Breast and Ovarian Guideline Panel) review the enclosed data and include more discussion of the benefits of multi-gene panel testing in addition to the existing discussion of caveats and limitations.

Specific Changes:
GENE-1 (Now page EVAL-A 3 of 6)
1) Change bullet 2 from "Patients who have a personal or family history suggestive of a single inherited cancer syndrome are most appropriately managed by genetic testing for that specific syndrome. When more than one gene can explain an inherited syndrome, then multi-gene testing may be more efficient and/or cost-effective" to "Patients who have a personal or family history suggestive of a single inherited cancer syndrome are most appropriately managed by genetic testing for that specific syndrome. However, there is extensive clinical and genetic overlap in hereditary breast or ovarian cancer. Therefore, multi-gene testing may be more efficient and/or cost effective as it allows for comprehensive assessment of clinically-actionable hereditary breast and ovarian cancer genes."
2) Add bullet as follows: Studies indicate that use of multi-gene testing leads to the detection of pathogenic variants in clinically actionable genes beyond a patient's initial presentation, identification of individuals with multiple pathogenic variants, and an increase in diagnostic yield.

FDA Clearance: N/A

Rationale: The current discussion on page GENE-1 focuses on the caveats and limitations of multi-gene panel testing (MGPT), which are valid and crucial in the test selection and interpretation process. However, the growing body of evidence in recent years has consistently demonstrated the benefit of MGPT as well. Furthermore, as these guidelines pertain to risk assessment for breast and ovarian cancer, which have shown to be particularly genetically heterogeneous, further insight from this expert panel on the use of MGPT will be especially helpful for health care practitioners.

Extensive data has been published illustrating that patients suspected of hereditary cancer syndromes have pathogenic variants (PVs) in clinically actionable genes beyond their primary testing indication. Myriad Genetics reported the outcomes of 252,223 patients tested with a 25-gene pan-cancer panel that met testing criteria for hereditary breast and ovarian cancer (HBOC) syndrome and/or Lynch syndrome (LS). Among patients who only met HBOC testing criteria, half (49.2%) of the PVs were in non-BRCA1/2 genes, with 6.5% PVs found in LS and colorectal cancer genes[1]. Similar results were found at Ambry Genetics; in an assessment of patients tested for 32 cancer predisposition genes (n = 33,987), two thirds of PVs in patients meeting criteria for *BRCA1/2* occurred in other genes. Specifically, 53.9% of PVs occurred in other breast and/or ovarian cancer genes, 5.2% in Lynch syndrome genes, and 7.8% in other cancer predisposition genes[2]. Data has also shown that a significant portion of individuals (up to 53% in

ovarian cancer patients) with PVs in clinically actionable genes have no personal or family history consistent with their genetic diagnosis at all, which reinforces the fact that strict gene selection based on initial clinical presentation can fail to identify the source of risk in a patient or family[1]. To further illustrate this point, when the mutation profile of HBOC cases from nine MGPT studies is curated, (overall N=112,863; PVs carriers=21,111) totals indicate that restricting testing to *BRCA1/2* misses half of individuals with PVs, with 48% of individuals carrying *BRCA1/2* PVs, 42% carrying moderate-risk HBOC PVs, and 7% of individuals with PVs in LS or polyposis genes [1,3-10].

In addition, it has been well established that cancer types can have several cross indications and be suggestive of more than one gene. For example, individuals with epithelial ovarian cancer are at risk to carry a mutation in HBOC genes such as *BRCA1/2* or in a LS gene. In one study of 7768 clinically ascertained individuals with ovarian cancer, 43% of PVs were identified in genes other than *BRCA1/2*, including 10% in LS genes and 33% in other known/suspected ovarian cancer susceptibility genes[11]. Likewise, between 41-45% of PVs identified in pancreatic cancer patients are in *BRCA1/2*, 6-18% are in LS genes, and 41-45% are in other pancreatic cancer susceptibility genes[12,13].

Lastly, adoption of MGPT has brought to light the phenomenon of multiple mutation carriers, in which individuals harbor PVs in more than one actionable gene. In Ambry's cohort of 165,000 individuals undergoing MGPT, up to 3.3% of individuals had PVs in more than one gene[2]. This observation has been reproducible, with multiple mutations accounting for 2-4.5% of positive individuals[1,14].

These findings all illustrate the benefits that MGPT can provide in the identification of patients with hereditary cancer syndromes and help explain why clinicians increasingly utilize MGPT as a first line of panel testing. In light of recently emerged data, a balanced discussion of the benefits and limitations of MGPT, and one that is in alignment with current practice in a hereditary breast/ovarian cancer clinical setting, is warranted and appropriate.

Citations:

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11) Lilyquist J, LaDuca H, Polley E, et al. Frequency of mutations in a large series of clinically ascertained ovarian cancer cases tested on multi-gene panels compared to reference controls. *Gynecol Oncol.* 2017;147(2):375-380.
12) Dudley B, Karloski E, Monzon FA, et al. Germline mutation prevalence in individuals with pancreatic cancer and a history of previous malignancy. *Cancer*. 2018.
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