Title: **Pathogenic variants among female breast cancer patients with a subsequent cancer demonstrate preventable cancer burden.**

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Background: Among females with breast cancer, multiple primary cancers (MPCs) are a hallmark of cancer predisposition syndromes. Herein we report the frequency of germline pathogenic/likely pathogenic variants (PVs) among females with breast cancer
(BC) and primary cancer of >1 other site who underwent multi-gene panel testing (MGPT) through a single lab. Among females first diagnosed with BC who had a PV in an actionable gene, we quantified the frequency of subsequent breast and non-breast cancers. Among those who later developed a second BC, ovarian, endometrial or colon cancer, we determined the percentage of patients with MPCs who would have been identified as high-risk if all women had had MGPT after a first diagnosis of breast cancer.

Methods: Females with breast cancer and MPCs who underwent germline genetic testing with Ambry Genetics from 3/2012 to 12/2016 were included in our cohort. Eligible individuals had multigene panel testing, which included 21 genes at minimum (ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, and TP53). Clinical factors including age at diagnosis, age at testing and cancer type were obtained from test requisition forms and clinical notes. Patients with >1 PV were excluded from the analysis.

Results: Of the 6617 patients with MPCs including BC tested for the 21 genes in the analytic cohort, most were white (70.8%), with median age at testing 63 years (IQR: 16). The median ages of first and second cancer diagnosis were 49 (IQR: 18) and 59 (IQR: 16) years, respectively. Considering the diagnostic order of BC, PV prevalence was 16.5% (444/2687) with BC as the 1st cancer diagnosis, 11.2% (296/2641) with BC as the 2nd cancer diagnosis, 15.2% (49/323) as 3rd or later diagnosis ($p<0.001$). Based on
the PV identified through genetic testing, 411 of 2687 (15.3%) females with BC as a first diagnosis would have been identified as high-risk and 227 (8.4%) as eligible for altered surveillance or risk-reducing interventions for a cancer that they subsequently developed. Specifically, of the 318 (11.8%) females with a PV in ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PTEN, STK11, or TP53, 73 (23%) developed a subsequent BC. Of the 241 (9.0%) females with PVs in genes including ovarian cancer susceptibility

(BRCA1, BRCA2, BRIP1, PALB2, RAD51C, or RAD51D), 143 (59.3%) developed ovarian cancer, 21 of whom also developed a second breast cancer. Among the 41 (1.5%) females with Lynch syndrome, 25 (61.0%) were later diagnosed with endometrial cancer and 10 (24.4%) were diagnosed with colorectal cancers (3 had both).

Conclusions: Our data show a significant prevalence of germline PV in cancer susceptibility genes in women with MPC including BC. Further, the data suggest that a strategy testing all BC patients at diagnosis to identify actionable PV could permit the appropriate use of risk-reducing and surveillance strategies to reduce the occurrence of MPC among BC survivors.