Title: Characterizing the mutation spectrum in *BRCA1/2*-negative early-onset breast cancer patients undergoing multigene panel testing

Background: Women diagnosed with early onset breast cancer often seek knowledge from genetic testing for informed decision making with regards to treatment and prevention. National Comprehensive Cancer Network guidelines support *BRCA1/2* testing for early-onset breast cancer, as one of their testing criteria is breast cancer diagnosed at age 45 or younger. As multigene panel testing becomes more commonplace, clinicians are able to test for other genes associated with hereditary breast/ovarian cancer (HBOC). As such, we aim to define the mutation spectrum in *BRCA1/2* mutation-negative women diagnosed with breast cancer at age 45 or younger.

Methods: Results from HBOC multigene panel testing, performed at our clinical diagnostic laboratory, were retrospectively reviewed for a cohort of 4066 *BRCA1/2* mutation-negative patients diagnosed with breast cancer at age 45 or younger. Panels included comprehensive analysis of 6-23 genes, depending on the test ordered (BRCAplus, BreastNext, or OvaNext). Mutation frequencies were calculated for the following breast and/or ovarian cancer genes: *CHEK2, ATM, PALB2, TP53, BARD1, RAD50, NBN, PTEN, MRE11A, RAD51C, BRIP1, RAD51D, NF1*, and *CDH1*. Mutation frequencies were calculated based on the number of times each gene was sequenced in this cohort and then compared with those observed in 4615 breast cancer patients diagnosed after age 45.

Results: In this cohort, 160 (4%) patients diagnosed at age 45 or younger were positive for at least one of 14 genes analyzed in this study. Eight (0.2%) patients were positive for more than one mutation in the genes analyzed. The most commonly mutated gene in this cohort was *CHEK2* (4.10%) followed by *ATM* (1.6%), *PALB2* (1.15%), and *TP53* (0.81%). Mutation frequencies for each of the remaining 10 genes was <0.50%. Mutations in *ATM*, *CHEK2*, and *TP53* were significantly more frequent among patients diagnosed age 45 or younger when compared to women diagnosed after age 45 (p-value <0.05).

Conclusions: This study demonstrates that *CHEK2*, *ATM*, *PALB2* and *TP53* are the most commonly mutated genes among *BRCA1/2* mutation-negative early-onset breast cancer patients. Given the frequency of these mutations in this younger population, molecular diagnosis for these genes may be important. More research is needed to validate these results and better understand the utility of identifying these mutations for cancer treatment and prevention, in the hopes of developing evidence-based guidelines for testing beyond BRCA1/2.