BRCA1 and *RAD51C* Promoter Hypermethylation Confer Sensitivity to PARP Inhibitors in Patients with Platinum Sensitive Ovarian Carcinoma

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Abstract

Germline and somatic mutations in *BRCA1* and *BRCA*2 (BRCA) confer PARP inhibitor sensitivity. Promoter hypermethylation is an alternate mechanism of gene down-regulation, and *BRCA1* promoter methylation is relatively common in sporadic ovarian cancer. The clinical significance of *BRCA1* methylation is less clear than for mutations, as the Cancer Genome Atlas (TCGA) and others have failed to show improved survival in ovarian carcinomas with *BRCA1* methylation. No one has previously tested whether *BRCA1* methylation confers *in vivo* sensitivity to PARP inhibitors in patients with ovarian cancer. ARIEL2 is a phase 2 study of the PARP inhibitor rucaparib in patients with recurrent platinum sensitive high-grade ovarian, peritoneal or fallopian tube carcinoma. At enrollment, ARIEL2 required pre-treatment tumor biopsies with the goal of developing tissue predictors of PARP inhibitor sensitivity other than *BRCA* mutations. The number of women with known germline mutations was capped at 15 patients in order to predominantly enroll *BRCA* wildtype cases. As presented at ASCO 2016, in cases with no *BRCA* mutations, a high fraction of genomic loss of heterozygosity (LOH) significantly predicted a better progression-free survival (the primary endpoint), longer duration of response, and a higher fraction of responders compared to cases with low LOH. We assessed *BRCA1* and *RAD51C* promoter hypermethylation using methylation-sensitive polymerase chain reaction in paired archival and pre-treatment biopsies from patients on ARIEL2. Of 165 cases for which methylation analyses were completed, 21 (12.7%) were methylated at the *BRCA1* promoter and four (2.4%) at the *RAD51C* promoter. Methylation of *BRCA1* and *RAD51C* was mutually exclusive with mutation in *BRCA* or other homologous recombination genes. All four cases with *RAD51C* methylation and 15/19 (78.9%) with *BRCA1* methylation were associated with high LOH. In 90 paired samples archival and pre-treatment tissues, *RAD51C* methylation was 100% concordant and *BRCA1* methylation was highly concordant ($p<0{\cdot}001$). For 13 cases with *BRCA1* methylation in the archival specimen, 4 (30·8%) were unmethylated in the paired pretreatment tumor, but for 77 unmethylated archival specimens, gain of methylation in the pretreatment biopsy was observed just once. Confirmed RECIST responses were seen in 52.4% (11/21) *BRCA1* methylated and 75.0% (3/4) *RAD51C* methylated cases. In conclusion, *BRCA1* and *RAD51C* methylation in ovarian carcinomas correlates with a high response rate to PARP inhibitors. If methylation was to be used as a predictor of PARP inhibitor sensitivity, it would need to be assessed in a pre-treatment (not archival) specimen. The loss of *BRCA1* methylation in recurrent ovarian carcinoma, which was common even in these platinum sensitive cases, could explain why *BRCA1* methylation is

associated with similar survival to methylated cases, despite initial improved therapeutic sensitivity.