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CORRESPONDENCE

Functional HRD by RAD51 identifies *BRCA1* VUS associated with loss of gene function and response to DNA-damaging agents

Almost 15% of patients with breast and high-grade serous ovarian cancer (HGSOC) undergoing germline genetic testing harbor variants of uncertain significance (VUS) in *BRCA1* and *BRCA2* genes.[1] VUS represent a challenge both for risk assessment and prediction of response to treatments. Tools to assess the functional impact of VUS are needed to evaluate their predictive role of response to DNA-damaging agents.

RAD51 assay is a functional and dynamic test carried out on tumor tissue which reflects the homologous recombination repair (HRR) status.[2,3] The assay tests the immunofluorescence of γH2AX as sensor of double-strand break DNA damage, BRCA1 as one of the main HRR mediators, and RAD51 as HRR final effector. When HRR is proficiently activated, RAD51 forms nuclear foci, identifying the HRR-proficient (HRP) status[4]; the absence of nuclear foci identifies the HRR-deficient (HRD) status. We carried out RAD51 assay on tumor samples from three patients harboring the germline VUS c.4096+1G>A,[5] in heterozygous state, in *BRCA1* gene and treated with platinum-based chemotherapy and/or poly(adenosine diphosphate–ribose) polymerase (PARP) inhibitors (PARPi). All patients were tested for the presence of germline pathogenic variants in *BRCA1/2* and low penetrance genes, and somatic mutations in *BRCA1*/2, using next-generation sequencing on blood and tumor samples, respectively.[6] No germline pathogenic mutations were detected in the other genes.

The clinical history of the three patients is briefly reported:

- Patient number 1, 66-year-old, diagnosed with HGSOC, underwent surgery, first-line chemotherapy with carboplatin plus paclitaxel and maintenance treatment with bevacizumab. After 2 years, she relapsed and was treated with carboplatin plus gemcitabine with rapid progression of disease (PD).
- Patient number 2, 42-year-old, daughter of patient number 1, diagnosed with metastatic breast cancer, was treated with carboplatin with a rapid PD. She subsequently received olaparib through off-label use, with no response and PD.
- Patient number 3, 37-year-old, diagnosed with HGSOC, underwent chemotherapy with carboplatin plus paclitaxel in a perioperative setting with interval debulking surgery, obtaining pathological complete response. She started niraparib maintenance therapy and is currently progression-free after 3 years.

RAD51 assay was carried out on tumor samples collected before carboplatin treatment (Figure 1):

- Patient number 1: BRCA1 and RAD51 foci were detected, classifying sample as HRP.
- Patient number 2: BRCA1 and RAD51 foci were detected, classifying sample as HRP.
- Patient number 3: BRCA1 and RAD51 foci were absent, classifying sample as HRD.

To characterize the zygosity status on tissue samples, the germline variant *BRCA1* c.4096+1G>A was tested in the same tissue biopsy studied for RAD51 assay. All patients presented the VUS at variant allele frequency (VAF) consistent with a homozygous state (with a range of 94%-95.8%), suggesting loss of heterozygosity as a possible second hit.

In our case series, the only patient harboring *BRCA1* VUS and showing BRCA1 loss of function and HRD status according to RAD51 assay obtained a remarkable and durable response to carboplatin/PARPi.

Based on our results, we may speculate that RAD51 assay could successfully predict response to DNA-damaging therapies in patients harboring the VUS c.4096+1G>A in BRCA1 gene. Our work has some limitations: patients' clinical settings are different and do not permit a direct comparison of response to platinum salts and/or PARPi; furthermore, we cannot exclude that HRD status could be related with other mechanisms independent from VUS (e.g. BRCA1 promoter methylation).

The potential predictive role of RAD51 assay in patients with VUS in *BRCA1/2* and other HRR genes should be investigated in a wider population.

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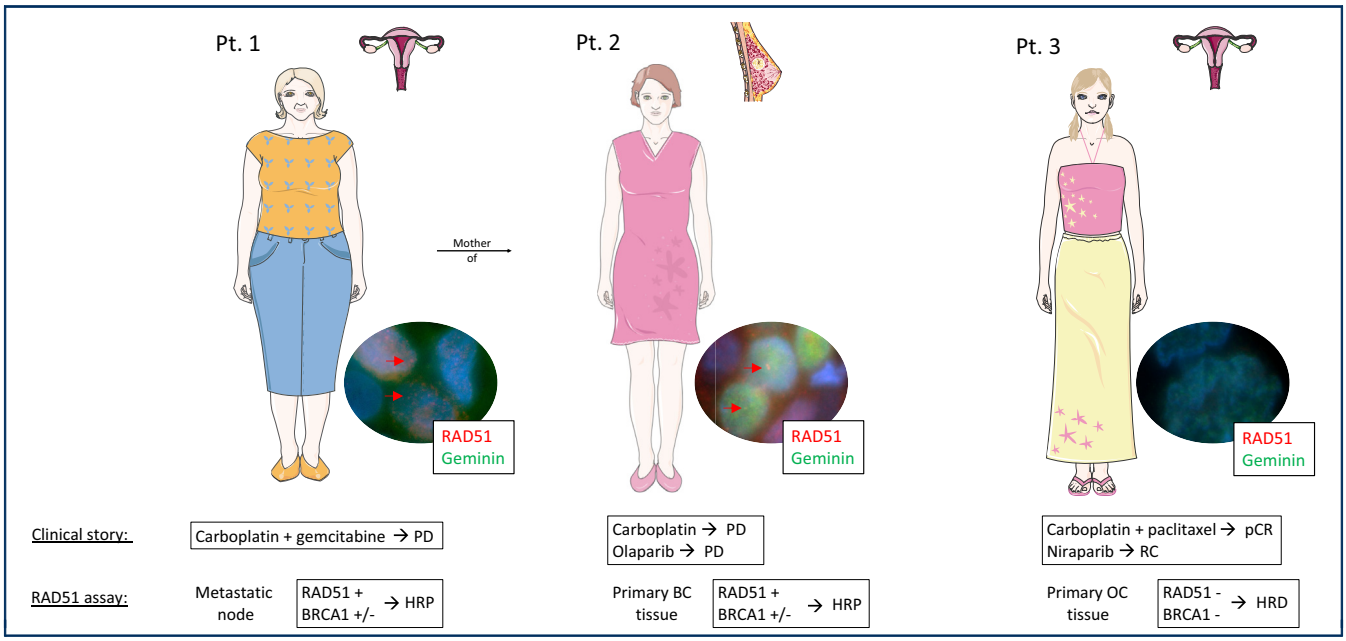

Figure 1. Graphical abstract of the results.
BC, breast cancer; HRD, homologous recombination repair-deficient; HRP, homologous recombination repair-proficient; OC, ovarian cancer; pCR, pathological complete response; PD, progression of disease; Pt., patient; RC, complete response

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