



43_Detection of somatic and germline BRCA1/BRCA2 mutations in ovarian carcinomas

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Aim: Ovarian cancer is the most common cause of death from gynecological tumors in the Western world. It is a heterogeneous disease, with high-grade serous ovarian cancer (HGSOC) being both the most common and the most aggressive histological subtype. In up to 50% of patients with HGSOC, the tumor cells may present homologous recombination deficiency as a result of germline or somatically acquired *BRCA1/BRCA2* mutations, epigenetic silencing of *BRCA1*, or defects in the homologous recombination pathway that are independent of *BRCA1/BRCA2*. Olaparib is a PARP inhibitor that is indicated, in the EU, as monotherapy for maintenance treatment of patients with platinum-sensitive, relapsed, BRCA-mutated (germline or somatic), HGSOC, fallopian tube, or primary peritoneal cancer. In this study we aimed to test the feasibility of analyzing ovarian FFPE tumor tissue for *BRCA1/BRCA2* mutations by next-generation sequencing (NGS).

Material and Methods: This study included a total of 60 ovarian FFPE tumor samples, 10 of them with a known germline pathogenic *BRCA1/BRCA2* mutation (positive controls) and 50 with unknown *BRCA1/BRCA2* mutational status. The *BRCA2* c.156_157insAlu and the *BRCA1* c.3331_3334del Portuguese founder mutations were tested prior to tumor analysis whenever peripheral blood DNA was available. FFPE samples were analyzed using the BRCA Tumor MASTR Plus assay (Multiplicom) that provides *BRCA1/BRCA2* full coverage of all exons and adjacent intronic regions. All mutations identified were confirmed by Sanger sequencing in tumor tissue and peripheral blood DNA was tested when available to determine if the mutations were somatic or germline. The *BRCA2* c.156_157insAlu mutation was systematically screened in peripheral blood DNA.

Results: We were able to identify *BRCA1/BRCA2* mutations in 8 of the 10 positive controls, with the only exception being the *BRCA2* c.156_157insAlu, as expected due to the mutation type. In one of the FFPE tumor samples, DNA was of insufficient quality to perform NGS. In the remaining 49 samples with unknown *BRCA1/BRCA2* status, we identified a total of eight pathogenic *BRCA1/BRCA2* mutations (16.3%), with seven being germline and the remaining somatic. Founder mutations represented 50% (4/8) of the pathogenic mutations identified: three samples with the *BRCA1* c.3331_3334del mutation and one with the *BRCA2* c.156_157insAlu mutation.

Conclusions: In this study, we show that *BRCA1/BRCA2* mutation analysis is feasible in DNA extracted from FFPE tissue using NGS. All germline point mutations of the positive controls were identified in tumor tissue, with the *BRCA2* c.156_157insAlu mutation being detectable in peripheral blood DNA. Additionally, we identified pathogenic mutations in 16.3% of the samples with previously unknown *BRCA1/BRCA2* mutational status, making possible the



evaluation of indication for PARP inhibition therapy. Considering that 50% of the pathogenic mutations identified were one of the Portuguese founder mutations and that somatic mutations represent 12.5% of all deleterious mutations, we recommend that founder mutations should be initially screened in peripheral blood DNA samples, prior to tumor analysis by NGS. Finally, if not tested earlier, tumor NGS analysis should be complemented with screening of the *BRCA2* c.156_157insAlu mutation in germline DNA.