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Case report

Can *TP53* variant negative be high-grade serous ovarian carcinoma? A case series

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1. Introduction

Ovarian cancer (OC) accounts for the second leading cause of gynecologic malignancies worldwide with high-grade serous ovarian carcinoma (HGSOC) being the most common subtype (Torre et al., 2018). Tumor *TP53* variants are an important hallmark of HGSOC with nearly all being *TP53* variant positive (Network CGAR, 2011), and can potentially guide disease monitoring and treatment response on circulating tumor DNA (ctDNA) (Parkinson et al., 2016). However, careful review of integrated genomic data from The Cancer Genome Atlas (TCGA) study reveals that ~ 4% of HGSOC are *TP53* variant negative (Network CGAR, 2011). Historically, immunohistochemistry (IHC) has been used for the detection of p53 abnormalities in OC and is nearly 100% predictive of tumor *TP53* mutational status, leading to routine use in diagnosis (Köbel et al., 2016). p53 IHC expression falls under the following categories: overexpression, complete absence, cytoplasmic or wild type (Köbel et al., 2016). However, IHC cannot identify specific *TP53* mutations and thus cannot be used for monitoring. It is important to understand alternative approaches considered in the absence of *TP53* variants in HGSOC.

At our Centre, we initiated the Biomarker Discovery Project in High Grade Serous Ovarian Cancer (BioDIVA, NCT03419689), through which genomic profiling data is collected for each subject with a histologically confirmed HGSOC diagnosis. Tumor profiling for enrolled subjects was completed in a College of American Pathologists/Clinical Laboratory Improvement Amendment (CAP/CLIA)-accredited laboratory using a multigene targeted panel spanning exonic regions of 555 cancer-related genes (UHN Hi5 Panel) (Lheureux et al., 2018). Germline testing using 23-gene panel comprised of genes related to high-risk breast and/or ovarian cancers was also performed routinely through the Familial Cancer Clinic.

In this case series, we describe the histopathological, immunohistochemical and genetic attributes of three *TP53* variant negative cases identified in BioDIVA, which raise questions regarding diagnostic and therapeutic considerations in this subset of HGSOC.

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2. Case presentation 1

A 52-year-old was diagnosed incidentally from unilateral salpingo-oophorectomy, and underwent upfront debulking surgery confirming Stage IIIB HGSOC after central gynecology pathology review (Fig. 1A). p53 immunostains showed diffuse overexpression in the tumor (Fig. 2A-B), and positive PAX8 and WT-1. Pathogenic germline *BRCA1* variant (c.3342delA; p.Glu1115Lysfs*2) was detected and strong familial cancer history was noted. Tumor profiling from surgical specimen did not reveal *TP53* variant, but confirmed the presence of *BRCA1* variant, and rare *PARP1* variant (c.2905G > A; p.Val969Ile) of uncertain significance. The patient exhibited a serous tubal intraepithelial carcinoma (STIC) lesion (Fig. 2C), which also demonstrated diffuse p53 IHC overexpression (Fig. 2D) and moderately increased Ki67 proliferative index (Fig. 2E). She received adjuvant cisplatin and paclitaxel then maintenance olaparib, a Poly (ADP-ribose) polymerase (PARP) inhibitor, continuing beyond one year (Fig. 1A).

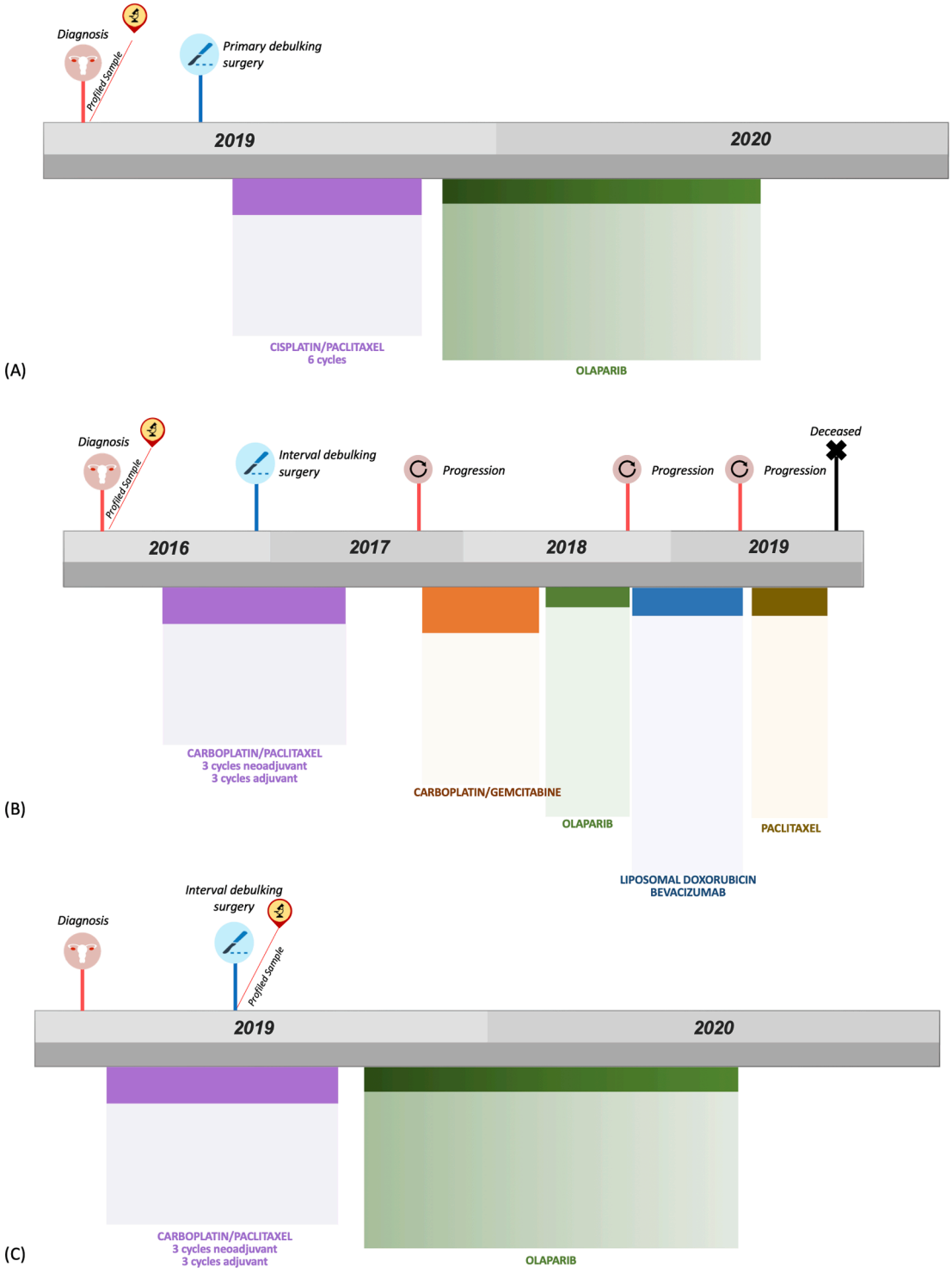

Fig. 1. Timeline describing oncological history of individual patient following initial diagnosis and throughout the course of treatment until current for A) Patient 1, B) Patient 2, and C) Patient 3.

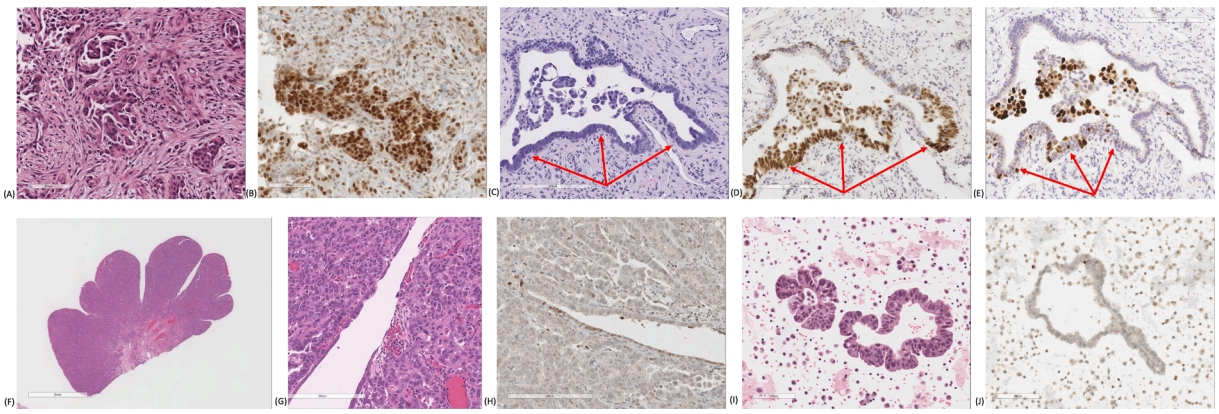

Fig. 2. Case 1 (A-E): High power H&E image of invasive high grade serous carcinoma in patient 1 showing typical morphologic features of slit-like spaces, high-grade cytologic atypia and brisk mitotic activity (2A) with corresponding mutant overexpression of p53 IHC (2B); high power H&E image of STIC lesion (with HGSC fragments present within the luminal space) (2C) with corresponding mutant overexpression of p53 IHC (2D) and moderately increased proliferative index by Ki67 (2E). Case 2 (F-H): Low power H&E image of the fimbriated end of the fallopian tube in case 2 distended by invasive high grade serous carcinoma (2F), with a higher power H&E image demonstrating tubal STIC lesion with subjacent invasion (2G) and corresponding null-type p53 IHC expression (2H). Case 3 (I-J): High power H&E image of metastatic high grade serous carcinoma in ascitic fluid cell block in case 3 (2I) with corresponding null-type p53 IHC expression (2 J).

3. Case presentation 2

A 48-year-old was diagnosed with stage IIIA HGSOC on omental biopsy, arising from the right fallopian tube associated with STIC lesion (Fig. 2F). p53 immunostaining on central gynecological pathology review showed null-type staining in both invasive tumor and STIC (Fig. 2G-H), and expression of PAX8 and WT-1. She received neo-adjuvant carboplatin and paclitaxel, underwent interval debulking to no residual disease, and completed adjuvant chemotherapy. Subsequently, she developed recurrent disease and was treated with multiple lines of chemotherapy and olaparib, and passed away nearly three years from date of diagnosis (Fig. 1B). Tumor profiling indicated absence of *TP53* variant, and presence of a rare *PARP1* variant (c.172 T > C; p. Trp58Arg) of uncertain significance. Germline panel testing results were negative, and family history was notable for lung cancer in her father and aunt.

4. Case presentation 3

A 51-year-old was diagnosed with stage IIIC high-grade serous peritoneal carcinoma on omental biopsy (Fig. 2I). IHC on both specimens was confirmatory of serous Müllerian tumor (PAX8 and WT-1 expression), and p53 staining was null-type (Fig. 2J). She received neo-adjuvant carboplatin and paclitaxel, interval debulking to no residual disease then further chemotherapy followed by maintenance olaparib, which continues beyond one year. She harbors pathogenic germline *BRCA1* variant (c.1480C > T, p.Gln494*). Tumor profiling from surgical specimen identified the germline *BRCA1* variant and did not detect *TP53* variants (Supplementary Table 1). She has no familial cancer history.

5. Discussion

A wide variety of clinical, imaging and laboratory parameters inform

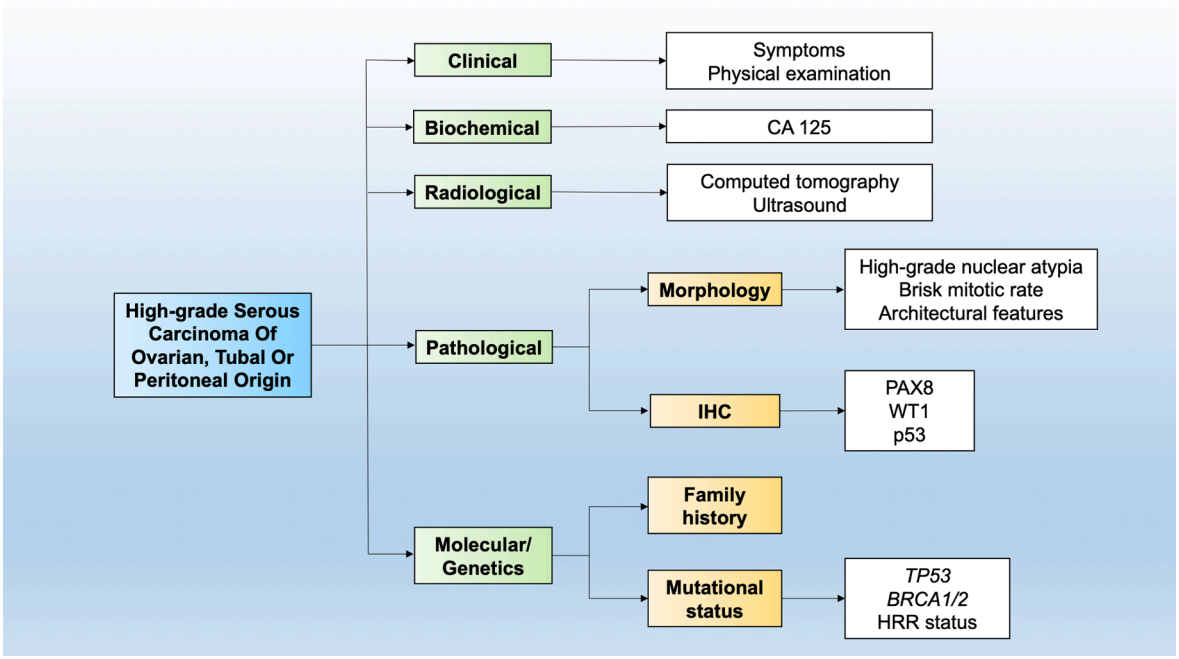

Fig. 3. Schematic for diagnostic considerations required for accurate diagnosis of HGSOC.

the diagnosis of HGSOC (Fig. 3). *TP53* variants are the most frequent genomic alterations in human cancers leading to loss-of-function, dominant-negative or gain-of-function phenotypes, and thus lead to carcinogenesis, metastasis and development of chemo-resistance (Zhang et al., 2016). True *TP53* variant negative HGSOC is a rare phenomenon, and absence of *TP53* variants through NGS poses a diagnostic dilemma which should lead to critical re-evaluation of all considerations for a conclusive diagnosis. In this case series, we shed light on the significance of each of these factors in aiding with confirmation of final pathological diagnosis of this rare subset of HGSOC.

5.1. p53 by immunohistochemistry or NGS

Abnormal p53 IHC expression is a robust surrogate marker for *TP53* mutations (Köbel et al., 2016) and is routinely used in diagnosing HGSOC as recommended by the World Health Organization (Köbel et al., 2016; Carcangiu et al., 2014). However, in each of the cases detailed above, abnormal p53 IHC was observed despite the lack of *TP53* mutation identified by tumor profiling, and outcomes of this particular subgroup have not been explored previously. One crucial limitation of tumor specimens is that they only allow pathological and genomic assessment at a single time point, which is affected by quality of samples assessed and tumor heterogeneity. In our case series, tissue biopsies sequenced from two of the three cases reported here met optimal tumor cellularity requirements (>20% tumor cellularity, to detect mutations to 3–5% variant allele frequency) for NGS assays, whereas one was below the standard cellularity accepted (5–10%)(Supplementary Table 1). Unfortunately, in all three cases presented, tissue sampling was not adequate to perform repeated sequencing, which would have been especially useful in the case where cellularity was below standard. NGS assays only detect *TP53* mutations in regions covered by the assay (in this case, exonic regions and 5–10 bp of flanking intronic regions), and use of short-read NGS methods may not detect rare *TP53* variants, such as large insertions or deletions. Conversely, although IHC correlates well with NGS (Köbel et al., 2016), the tests differ in their sensitivities and specificities, and occasionally leads to false positives. In some tumors aberrant p53 expression may also be linked to alterations in p53 regulators such as MDM2, p14-ARF and other molecular chaperones that control stabilization (Xue et al., 2019; Wang et al., 2005). Using a combination of both assays is suggested for the most accurate classification; however, the dilemma remains as to which results are prioritized when discrepancies are present. (Köbel et al., 2016). Further investigation could be pursued through whole exome or genome sequencing. Although IHC may be more relevant for diagnostic characterization of HGSOC, knowledge of *TP53* mutation status is required for disease monitoring purposes using ctDNA. Even though the clinical implications of knowing the exact *TP53* variant status is limited currently, in a recent report by Oza *et. al.* differential clinical benefit by Adavosertib (AZD1775) Plus Paclitaxel and Carboplatin combination in women with HGSOC was observed with different *TP53* mutation subtypes (Oza et al., 2020).

5.2. STIC lesions

The presence of STIC lesions, optimally diagnosed through sampling via the SEE-FIM (Sectioning and Examining Extensively the FIMbriated end) protocol may be indicative of HGSOC diagnosis (Medeiros et al., 2006), as several studies have indicated they are precursory and arise from tubal epithelium years prior to malignant transformation (Zhang et al., 2019). Genomic assessments of STIC lesions consistently demonstrate that *TP53* variants are early driving events for HGSOC, although some differences in p53 status between precursor and carcinoma lesions may exist due to evolutionary changes and clonal diversity (Zhang et al., 2019). In our study, two cases demonstrated STIC lesions in histopathological specimens (Fig. 2), which showed identical mutant staining patterns on IHC compared with the accompanying invasive tumors.

5.3. BRCA mutations

The presence of *BRCA1/2* variants, together with loss-of-heterozygosity of the wild-type *BRCA1/2* allele, work synergistically with dysfunctional p53 pathways causing carcinogenesis (Sowter and Ashworth, 2005). Although the presence of a *TP53* variant is ubiquitous in *BRCA*-mutated HGSOC, careful review of large genomic datasets from AACR-GENIE, TCGA and other studies (Boyarskikh et al., 2020) showed a small subset of patients without *TP53* variants but *BRCA1/2* variant positive (Supplementary Figs. 1 and 2). Additionally, one report of 25 *TP53* variant-negative HGSOC cases highlighted nine cases with homologous recombination repair pathway abnormalities (Chui et al., 2020). In our series, two patients were *TP53* variant negative with pathogenic germline *BRCA1* variants. This observation is similar to recent reports (Boyarskikh et al., 2020) in *BRCA1/2* mutant HGSOC, however no *CDKN2A* variants were seen.

Amongst other mutational events, it is interesting to note that two patients exhibited presence of rare *PARP1* variants, classified as variants of uncertain significance. PARP1 proteins are involved in processes such as DNA repair, replication, transcription and chromatin remodeling (Jiang et al., 2015), and mutations have been linked to altered sensitivity towards PARP inhibitors (Pettitt et al., 2018). In the patients described, the presence of *PARP1* variants did not appear to affect response to PARP inhibition. The predictive role of *PARP1* variants in the context of *TP53* variant negative HGSOC is yet to be established.

In conclusion, this report summarizes three cases of HGSOC with abnormal p53 IHC, without *TP53* variants on tumor molecular profiling, examining exonic regions. Our findings support that contrary to *TP53* mutational status, p53 immunostaining patterns along with morphological parameters are more robust in distinguishing serous carcinomas as low-grade versus high-grade. This study highlights the importance of using a combination of NGS and non-NGS assays in assessing the p53 functional status for clinical trial inclusion, as well reviewing other evaluators in order to overrule the possibility of misdiagnosis. This may include: 1) re-evaluation of clinico-pathological features; 2) genetic re-assessment through more comprehensive assays, such as whole genome and ctDNA analysis (Patch et al., 2015; Imperial et al., 2019) to ensure no missed rare or complex genomic variants, preferably on different tissue samples; and 3) ensuring optimal quality and quantity of tumor sample is used. This study also emphasizes the difficulty in relying on p53 abnormality as the sole diagnostic marker in HGSOC, since some tumors which are morphologically HGSOC (approximately 4%) may have wild-type p53 staining pattern (Köbel et al., 2016). Most interestingly, this study highlights the importance of identification of additional genomic variants in HGSOC patients where *TP53* variants are not detected by NGS. The information on *BRCA1* and/or *PARP* variants if available upfront at the onset of treatment, could influence the treatment plan. Two of the three patients in our study carried *PARP* variants, and therefore, might not have been treated with PARP inhibitors. These findings are important as that may lead to the development of a customized panel that will define an increasingly refined approach to determining unique molecular signatures for HGSOC. Lastly, this study emphasizes reconsidering diagnosis of HGSOC when tumors are *TP53* variant negative by NGS and/or p53 IHC wild type.

6. Author contributions**a

Study conception and design was performed by LK, SG and SL; Study supervision by SL, ND and AMO; funding acquisition performed by SL, AMO and KK; Data curation, analysis and manuscript writing by LK and SG; pathology review was performed by BC and NT; genomic testing was done under the supervision of TLS; germline review was performed by RK. Final review was done by all authors.

7. Consent**a

Written informed consent was obtained from the patients as part of the BioDIVA study (NCT03419689), which was approved by the University Health Network Research Ethics Board, for collection of tumor tissue and blood for genomic profiling as well as publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2021.100729.

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