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Angiogenic burst after PARP inhibitor maintenance therapy in relapsed ovarian cancer: a case report

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ABSTRACT

In the post-PARP inhibitor era, potential changes in tumor biology after maintenance therapy have not been well investigated in recurrent ovarian cancer. We reported a case with alterations in the clinical and histological features of multiple relapsed disease associated with PARP inhibitor maintenance therapy. The patient with high-grade serous carcinoma exhibited *BRCA* wildtype and homologous recombination proficiency status, and suffered from three recurrences and surgeries accordingly. Olaparib maintenance had been used during the second-line therapy. We compared the differences in clinics and pathology among three recurrences and relapsed lesions. Disease-free survivals were dramatically decreased after the exposure to olaparib. At exploration of quaternary cytoreduction, the relapsed tumor was characterized by a carcinomatosis-like metastasis pattern and an easy tendency of bleeding. Tumor cytopathological changes and alterations were observed in both the tumoral and non-tumoral stroma, among relapsed tumor tissues derived from secondary, tertiary and quaternary cytoreduction. Histopathology indicated hemorrhage, necrosis, atypical tumor cells, massive angiogenesis, and decreased CD8+ tumor-infiltrating lymphocytes, particularly in the third relapsed disease. To our knowledge, this is the first report to show a unique metastatic pattern of angiogenic burst after PARP inhibitor maintenance therapy in ovarian cancer, which seemed to trigger invasive tumor growth and immune suppression. Further prospective studies and translational research focusing cytoreductive surgery after PARP inhibitor could progressively lead to an understanding of the biological behavior and metastatic patterns.

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INTRODUCTION

Although the combination of surgery, chemotherapy and targeted therapy presents a hallmark of ovarian cancer management, a multiple recurrent pattern remains the most challenging event in the natural history of this disease. Several retrospective studies showed that patients with prior use of PARP inhibitor had a poor response to the next platinum-based chemotherapy and a decreased median progression-free survival (PFS) [1,2]. The post-hoc analysis of SOLO2 trial showed that olaparib group had lower efficacy of subsequent chemotherapy than the placebo control group [3]. As expectation, the significantly increased PFS of olaparib did not translate to the final benefit of overall survival in the treatment of *BRCA1/2* mutated recurrent ovarian cancer as a whole in the SOLO2 trial [4]. Recently, Park et al. reported an increased trend of subsequent progression-free interval (PFS2-PFS1) in recurrent patients who underwent secondary cytoreductive surgery as the third-line therapy after olaparib maintenance [5]. However, such a possible benefit of surgery in olaparib group was not significant, and even much weaker than that in the control group without prior use of any PARP inhibitors [5]. Before PARP era, patient selection criteria for secondary cytoreduction have been well developed as the cornerstone of complete resection and survival benefit [6-8]. Do they still work in the PARP setting? Does a recurrent patient with an isolated lesion or long-disease-free interval [6] continue to survive longer after secondary cytoreduction under the circumstance of prior use of PARP inhibitor? Whether, when and how surgery should be performed is an entirely innovative topic in recurrent disease after PARP inhibitor maintenance. Genomic status like *BRCA* mutation and homologous recombination deficiency (HRD) might also weight up the biological metastasis pattern.

To our knowledge, potential changes of tumor biology after target maintenance therapy have not been well investigated during multiple recurrences of ovarian cancer. Here, we reported a high-grade serous ovarian cancer patient who suffered from multiple recurrences and characterized by a unique metastatic pattern of sustained angiogenesis after PARP inhibitor maintenance therapy, which seemed to trigger invasive tumor growth in clinics.

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CASE REPORT

We present the case of a 56-year-old woman with histologically proven primary ovarian cancer who suffered from three recurrences and four line therapies (**Figure 1**). She participated in the AICE trial [9] and L-MOCA trial [10], and provided a written informed consent. These two study protocols were both approved by the ethics committee of Zhongshan Hospital, Fudan University (Approval number: B2014-117, B2018-068R). This patient was first diagnosed with ovarian cancer in November 2012, with the initial serum CA125 level of 772 U/ml. Primary debulking surgery procedures included hysterectomy and bilateral salpingo-oophorectomy, resection of omentum, pelvic peritoneum, diaphragmatic peritoneum, tumor in hepatorenal recess and mesenteric tumor lesions. Histology showed a high-grade serous carcinoma of the ovary. The International Federation of Gynecology and Obstetrics (FIGO) stage was IIIC. After primary debulking surgery with no gross residual disease, she was randomized to the intraperitoneal chemotherapy group of the AICE trial [9]. The patient underwent dose-dense early postoperative intraperitoneal chemotherapy with four cycles of weekly cisplatin and etoposide followed by six cycles of intravenous carboplatin and paclitaxel. She tolerated the chemotherapy well and subsequently had no evidence of disease for more than 5 years.

In July 2018, she was diagnosed with first recurrence by positron emission tomography-computed tomography (PET-CT) imaging. The relapsed disease was characterized by a pattern of localized sites, including tumors in the lower spleen pole, descending colon and mesocolon. The iMODEL score [8] was 0.8 with the serum CA125 level of 6.9 U/ml and treatment-free interval of 62 months. Secondary cytoreductive surgery with a complete resection was achieved with splenectomy, small-bowel resection and large-bowel resection. Then, adjuvant chemotherapy with four cycles of intravenous carboplatin and docetaxel was performed along with subsequent olaparib maintenance therapy in L-MOCA trial [10].

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After 16.5 months' oral olaparib maintenance monotherapy (300 mg in two 150 mg tablets, twice daily), the second recurrence was diagnosed by enhanced computed tomography (CT) scan with solitary tumor that located in the left flank and lower abdomen close to iliac vessel. Similar with the first recurrence, serum level of CA125 was only 5 U/ml. Without any interval between the exposure to olaparib and tertiary cytoreduction, the patient received a complete resection of the solitary tumor followed by six cycles of intravenous carboplatin and docetaxel chemotherapy.

Unfortunately, she was suffered from the third recurrence after a 6-month treatment-free interval. PET-CT scan showed multiple relapsed diseases. Then, she underwent quaternary cytoreduction after the image-based evaluation of resectability. Surgical exploration indicated a carcinomatosis-like appearance with disseminated metastatic tumors on the surface of small bowel and mesenterium. An extensive dissection was performed, including left hemicolectomy, partial jejunectomy and ileostomy, partial resection of the left ureter and para-aortic lymphadenoectomy, as well as the removal of carcinomatosis by electronic devices with 500ml of estimated blood loss. Subsequently, she underwent five cycles of carboplatin and liposome doxorubicin with grade 3 thrombocytopenia.

METHODS

The patient was last followed up onsite by March 2022. She reported healthy on call and refused face to face visit and examination. We listed pre-operative imaging, the intraoperative situs and corresponding histological features before and during the three cytoreductive surgeries. The tumor tissue from secondary cytoreduction with matched blood sample has been detected by *BRCA* testing and homologous recombination deficiency (HRD) testing (3D Medicines, Inc., Shanghai, China).

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Haematoxylin-Eosin (H&E) and Immunohistochemistry (IHC) staining

For the comparably histological features, we selected the similar recurrence site, which was located in mesocolon descendens, among tissues from secondary, tertiary and quaternary cytoreduction. Serial sections of formalin-fixed, paraffin-embedded tissues were cut at 4 μm thickness for H&E and IHC staining. H&E staining was performed following the routine protocols. IHC staining used the following primary antibodies: mouse anti-human CD34 (clone M-0117, Long Island Antibody, Shanghai, China, 1:100) , mouse anti-human CD31 (clone 1A10, Leica, Newcastle, UK, 1:200), rabbit anti-human CD8 (clone ab4055, Abcam, Cambridge, UK, 1:200), mouse anti-human p53 (clone D0-7, Leica, Newcastle, UK, 1:500), rabbit anti-human Ki-67 (clone MIB1, DAKO, Carpinteria, CA, USA, 1:200) and mouse anti-human PD-L1 (clone 22C3, DAKO, Carpinteria, CA, USA), performed on an Autostainer Omnis system (DAKO, Carpinteria, CA, USA), along with positive and negative controls, according to standard automated protocols. All finished H&E and IHC slides were scanned with a NanoZoomer S360 Digital slide scanner (HAMAMATSU PHOTONICS (CHINA) CO., LTD.) and reviewed by two pathologists independently. Tumor infiltrating lymphocyte (TIL) estimates were scored following the international guidelines developed by the International Immuno-Oncology Biomarker Working Group [11].

RESULTS

The patient was identified with germline and somatic *BRCA1/2* wildtype, and the tumor tissue from secondary cytoreduction has been tested as genomic status of homologous recombination proficiency (HRP). The three rounds of recurrence were mainly characterized by intraperitoneal metastasis, but without a typical increasing level of serum CA125. Before olaparib maintenance, the patient had a 62-month treatment-free interval and was predicted to be benefit more from secondary cytoreduction based on the iMODEL score of 0.8. However, an 18-month treatment-free interval were observed after surgery and chemotherapy followed by olaparib maintenance, and an only 6-month interval thereafter, with disease progression (**Supplementary Table S1**). In addition, the numbers of relapsed

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sites differed widely among the three recurrences. At the same time of olaparib withdrawal, the tertiary cytoreduction was performed and solitary site was found at exploration thoroughly. Remarkably, quaternary cytoreduction showed that carcinomatosis-like disease with an easy tendency of bleeding were developed at the third recurrence. Such a swift progression of metastatic behavior challenged our practicing surgical experience.

We evaluated histological features, including tumor cytopathological changes and alterations of both tumoral and non-tumoral stroma, among recurrent tumor tissues from secondary, tertiary and quaternary cytoreduction. **Supplementary Table S2** lists the features we assessed for, with representative examples shown in **Figure 2**. All tumors exhibited a mutation-type labelling of p53 (no expression pattern). With the progression of disease, there were markedly atypism and increased mitosis in tumor cells. A histological signature of less mesenchyma and rare cancer-associated fibroblast invasion was shown in all three recurrences. While, neovascularization, hemorrhage and necrosis were gradually increased as the relapse worsens. As shown in metastatic lesions of the third recurrence, angiogenic burst and transformed vessels, accompanied by massive hemorrhage, were much impressive. It might, to a large extent, challenge the initial experience of the biologically metastatic behavior in recurrent ovarian cancer.

Consistent with H&E staining, the CD34 and CD31 staining showed not only a significantly increased trend of positive expression but also an abnormal transformation in vessels (**Figure 2, Supplementary Figure S1**). CD8-positive TILs were randomly scattered within the tumor region and showed a declined trend of expression after olaparib maintenance therapy. The expression of PD-L1 decreased from combined positive score (CPS) 40 to CPS 5 (**Supplementary Figure S1**). Although Ki-67 staining showed a high proportion of positive cells when first recurrence, a gradually increased trend seemed to trigger more invasive tumor growth in clinics (**Supplementary Table S2**).

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DISCUSSION

This patient was characterized by multiple recurrences and a negative genomic abnormality with *BRCA* wildtype and HRP status. To our knowledge, this is the first report to compare three recurrent surgeries before and after PARP inhibitor maintenance therapy. Before the exposure to olaparib, she was estimated as low-risk of complete cytoreduction with more than five years of treatment-free interval. However, a subsequent 18-month platinum-free interval with single site of recurrence was observed after olaparib maintenance, and an only 6-month interval with carcinomatosis spread thereafter, which clinically showed an aggravation of malignancy in a unique relapsed pattern. We addressed that the invisibly biological tumor cells might be underestimated during tertiary cytoreduction. Histologically, multiple rounds of relapsed lesions were characterized by an obviously increased trend of neovascularization and immune suppression. We previously observed a similar feature of angiogenic burst and hemorrhage after PARP inhibitor maintenance in another patient with germline *BRCA1* pathogenic mutation who received secondary cytoreduction at second recurrence (data not shown). Whether or when surgery could block tumor progression after PARP inhibitor exposure is a challenging topic. It is necessary to explore tumor evolution in both visible and molecular aspects according to genomic instability and *BRCA* mutation status.

It has been well-known that sustained angiogenesis is essential for tumor growth and metastasis. Without the vascular support provided by neovascularization, tumor cells would usually undergo apoptosis or necrosis. However, in this case, we observed extremely disseminated hemorrhage and necrosis in stroma along with the progression of disease. It might be due to a functional abnormality of the massive angiogenesis and transformed vessels, especially in relapsed lesions at third recurrence. Such a remodelling process is likely to be related to an exaggerated stress response and is therefore profoundly influenced by the tumor microenvironment. In turn, angiogenesis also plays a major role in immune suppression. Neovascularization and immune exhaustion could unlock the potentially

effective combination factors and drive immune suppression by directly suppressing immune effector cells [12], which is consistent with our findings.

During the past few years, more and more studies focused on the mechanism of resistance to PARP inhibitor. Multiple biologically informed strategies of PARP inhibitor combination were currently investigated to enhance efficacy. Lim et al. found that the inhibition of vascular endothelial growth factor (VEGF) receptor 3 led to decreased levels of BRCA1 and BRCA2, and restored chemosensitivity in *BRCA2* wildtype ovarian cancer cells [13]. In a randomized phase 2 trial, the combination of olaparib and cediranib (a highly potent inhibitor of VEGF receptor) was more efficient than single olaparib in patients with *BRCA* wildtype recurrent ovarian cancer [14]. It was reasonable that the inhibition of angiogenic progression might be associated with the sensitivity of chemotherapy and PARP inhibitor. Otherwise, similar with our results, neovascularization developed after the exposure to PARP inhibitor.

Several retrospective studies have demonstrated that patients with prior use of PARP inhibitor had a decreased response to the next platinum-based chemotherapy compared to those without prior use of PARP inhibitor [1,2]. So did the post-hoc analysis of SOLO2 trial [3]. We here reported a unique feature of clinical and histological alterations that needs attention along with tumor progression after PARP inhibitor maintenance therapy. Therefore, we recently revised the protocol of the ongoing SOC-3 trial to an umbrella design (clinicaltrial.gov Identifier: NCT03983226). The key object is to investigate the survival benefit of cytoreductive surgery combined with niraparib maintenance therapy in both the prior use and non-prior use of PARP inhibitor cohorts [15]. In the future, the results from the randomized phase II SOC-3 trial would be helpful to reveal the effect of PARP inhibitor exposure on survival benefit from complete cytoreduction. More prospective and translational evidence embedded with PARP inhibitor are warranted to elucidate the biological behavior and molecular mechanism of tumor metastasis, thus to guide the management of ovarian cancer in clinical practice.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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REFERENCES

1. Cecere SC, Giannone G, Salutari V, et al. Olaparib as maintenance therapy in patients with BRCA 1-2 mutated recurrent platinum sensitive ovarian cancer: Real world data and post progression outcome. *Gynecol Oncol.* 2020;156(1):38-44.
2. Rose PG, Yao M, Chambers LM, et al. PARP inhibitors decrease response to subsequent platinum-based chemotherapy in patients with BRCA mutated ovarian cancer. *Anticancer Drugs.* 2021;32(10):1086-1092.
3. Frenel JS, Kim JW, Berton-Rigaud D, et al. 813MO Efficacy of subsequent chemotherapy for patients with BRCA1/2 mutated platinum-sensitive recurrent epithelial ovarian cancer (EOC) progressing on olaparib vs placebo: The SOLO2/ENGOT Ov-21 trial. *Ann Oncol.* 2020;31(suppl 4):S615.
4. Poveda A, Floquet A, Ledermann JA, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a final analysis of a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* 2021;22(5):620-631.
5. Park J, Kim SI, Jeong SY, et al. Second-line olaparib maintenance therapy is associated with poor response to subsequent chemotherapy in BRCA1/2-mutated

epithelial ovarian cancer: A multicentre retrospective study. *Gynecol Oncol.* 2022;165(1):97-104.

6. Chi DS, McCaughty K, Diaz JP, et al. Guidelines and selection criteria for secondary cytoreductive surgery in patients with recurrent, platinum-sensitive epithelial ovarian carcinoma. *Cancer.* 2006;106(9):1933-1939.
7. Harter P, du Bois A, Hahmann M, et al. Surgery in recurrent ovarian cancer: the Arbeitsgemeinschaft Gynaekologische Onkologie (AGO) DESKTOP OVAR trial. *Ann Surg Oncol.* 2006;13(12):1702-1710.
8. Tian WJ, Chi DS, Sehouli J, et al. A risk model for secondary cytoreductive surgery in recurrent ovarian cancer: an evidence-based proposal for patient selection. *Ann Surg Oncol.* 2012;19(2):597-604.
9. Shi T, Jiang R, Yu J, et al. Addition of intraperitoneal cisplatin and etoposide to first-line chemotherapy for advanced ovarian cancer: a randomised, phase 2 trial. *Br J Cancer.* 2018;119(1):12-18.
10. Gao Q, Zhu J, Zhao W, et al. Olaparib Maintenance Monotherapy in Asian Patients with Platinum-Sensitive Relapsed Ovarian Cancer: Phase III Trial (L-MOCA). *Clin Cancer Res.* 2022;28(11):2278-2285.
11. Hendry S, Salgado R, Gevaert T, et al. Assessing Tumor-Infiltrating Lymphocytes in Solid Tumors: A Practical Review for Pathologists and Proposal for a Standardized Method from the International Immuno-Oncology Biomarkers Working Group: Part 2: TILs in Melanoma, Gastrointestinal Tract Carcinomas, Non-Small Cell Lung Carcinoma and Mesothelioma, Endometrial and Ovarian Carcinomas, Squamous Cell Carcinoma of the Head and Neck, Genitourinary Carcinomas, and Primary Brain Tumors. *Adv Anat Pathol.* 2017;24(6):311-335.
12. Rahma OE, Hodi FS. The Intersection between Tumor Angiogenesis and Immune Suppression. *Clin Cancer Res.* 2019;25(18):5449-5457.

 doi:10.20944/preprints202206.0266.v1

13. Lim JJ, Yang K, Taylor-Harding B, Wiedemeyer WR, Buckanovich RJ. VEGFR3 inhibition chemosensitizes ovarian cancer stemlike cells through down-regulation of BRCA1 and BRCA2. *Neoplasia.* 2014;16(4):343-353 e341-342.

14. Liu JF, Barry WT, Birrer M, et al. Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. *Lancet Oncol.* 2014;15(11):1207-1214.

15. Shi T. XL, Zhu J.,, Liu J., Zhang P., Wang H., Feng Y., Zhu T. SOC-3 trial, impact of cytoreductive surgery and PARP inhibitor in platinum-sensitive secondary recurrent ovarian cancer: update to the study protocol for a phase II, multicentre, randomised umbrella trial. *Journal of Gynecological Oncology.* 2022:accepted.

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FIGURE LEGENDS

Figure 1. Case present: multiple recurrences and treatment in detail with last follow-up time of March 2022.

Figure 2. Representative examples of the histological features changed after PARP inhibitor maintenance. A, B, C and D, E, F: Low-power and medium-power H&E view showing the gradually increased hemorrhage in secondary, tertiary and quaternary cytoreduction respectively. G, H and I showing a gradually decreased trend of CD8-positive expression in secondary, tertiary and quaternary cytoreduction. J, K and L showing the CD34 staining with a significant increase of angiogenic burst and transformed vessels.

Supplementary Table 1S. Clinical aspects within three rounds of recurrence.

Supplementary Table S2. Alterations of histological features within three rounds of recurrence.

Supplementary Figure S1. Representative examples on the alterations of CD31 and PD-L1 expression after PARP inhibitor maintenance. A, B and C showing CD31 staining with a significant increase of angiogenic burst and transformed vessels. D, E and F showing a gradually decreased trend of PD-L1 expression.

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Figure 1.

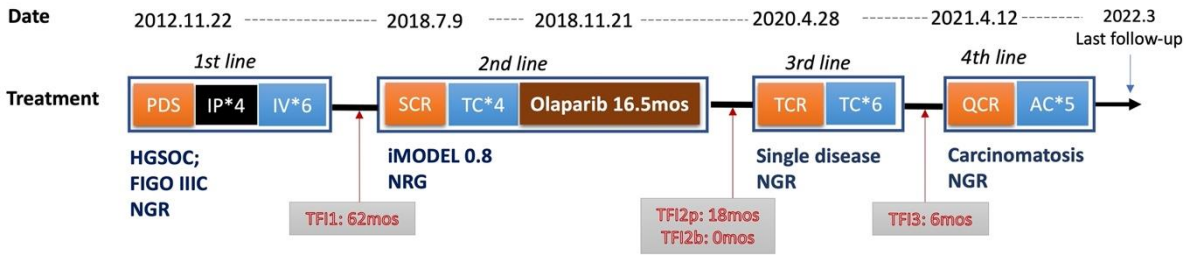
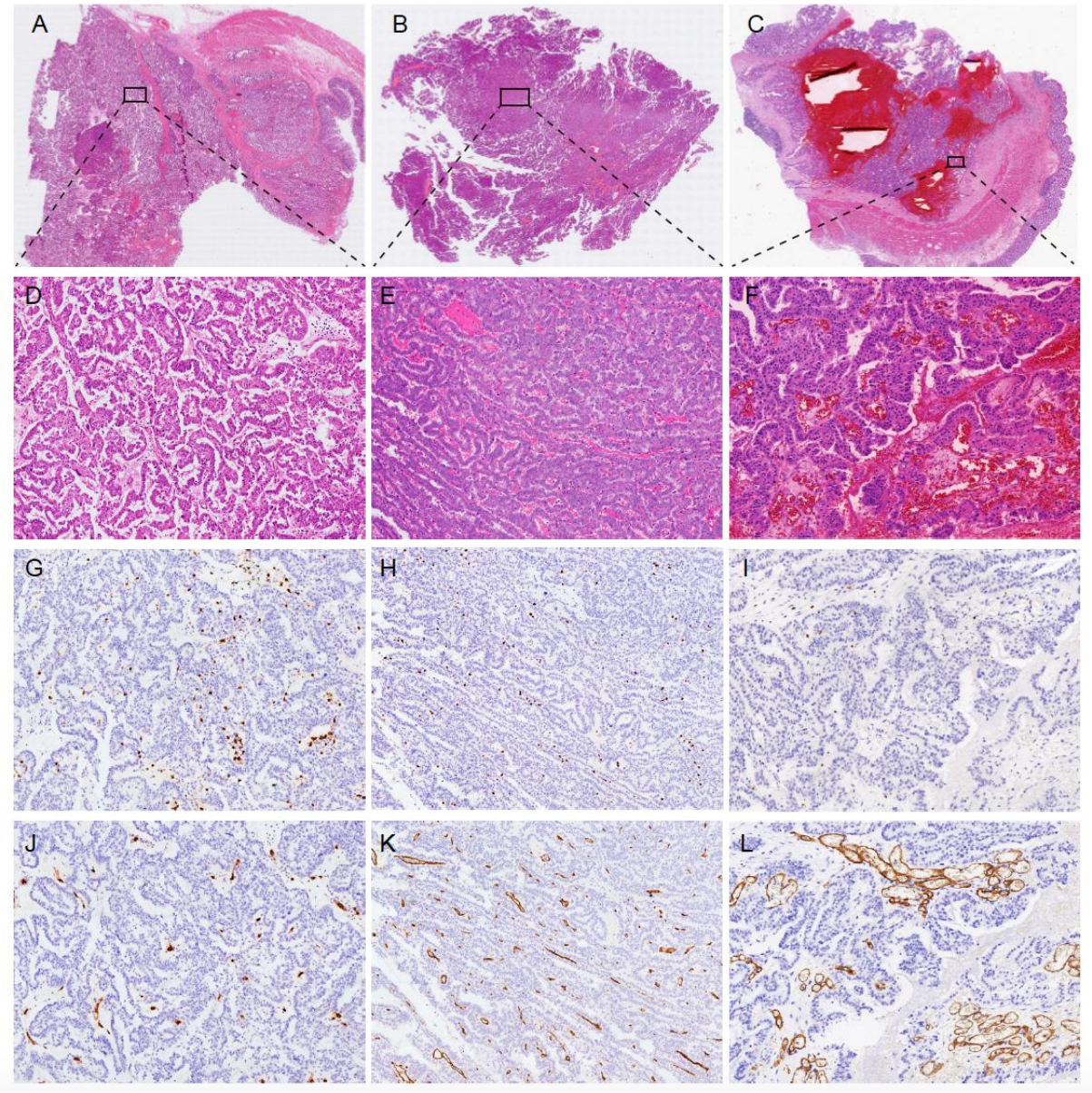


Figure 2.



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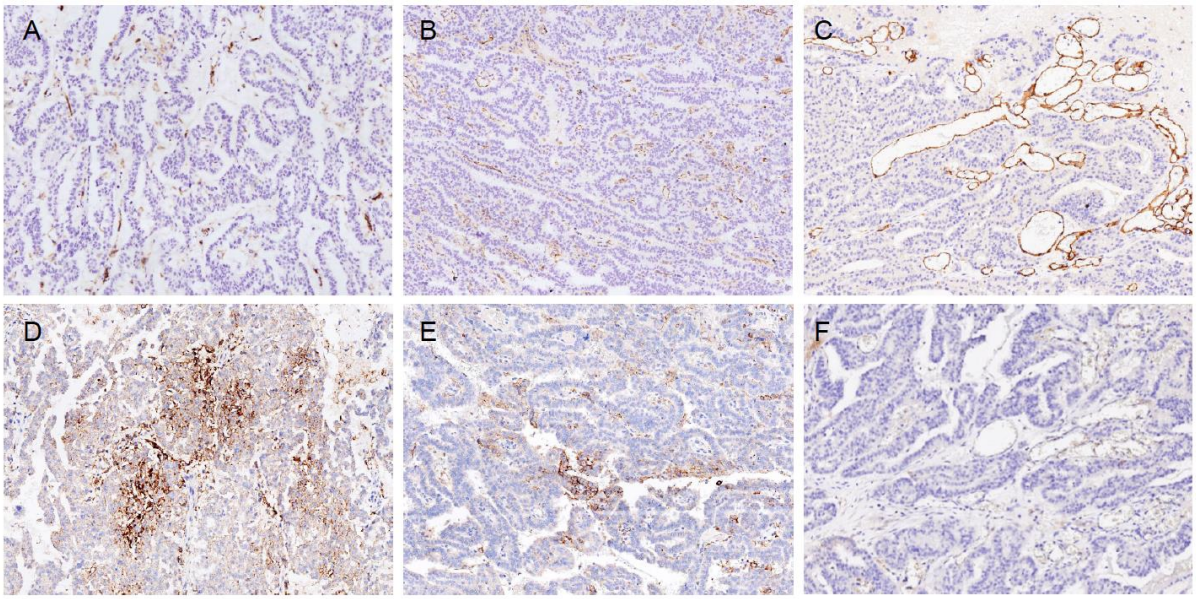
Supplementary Figure S1. Representative examples on the alterations of CD31 and PD-L1 expression after PARP inhibitor maintenance. A, B and C showing CD31 staining with a significant increase of angiogenic burst and transformed vessels. D, E and F showing a gradually decreased trend of PD-L1 expression.



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Supplementary Table S1. Clinical aspects within three rounds of recurrence.

	SCR in first recurrence	TCR in second recurrence	QCR in third recurrence
TFIp, mos	62	18	6
Serum CA125 at recurrence (U/ml)	6.9	5.0	11.1
Recurrent sites by imaging	Lesions in the lower spleen pole, descending colon and mesocolon	Tumor at left flank and lower of abdomen, close to the iliac vessels (50*54mm)	• Multiple tumor lesions adjacent to the descending colon (52*42mm, 31*27mm) with the dilatation of left ureter. • Mesenteric lymph nodes at the left lower quadrant (25*20mm). • Paraaortic lymph nodes (max 13*12mm).
Recurrent sites by surgical exploration	**Three sites:** • Tumor with diameter of 2cm in the lower spleen pole, membranous-like lesion covering the surface of spleen. • Two tumor lesions involved mesocolon descendens and small bowel.	**Single site:** Tumor with diameter of 5cm in mesocolon descendens, adjacent to the left ureter.	**Carcinomatosis:** • Tumor with diameter of 12cm at left paracolic gutter, which involves the descending and sigmoid colon, jejunum, duodenum, and the left ureter. • Two tumors involving the ileum wall and at the root of meso-ileum. • Multiple micronodules at the surface of the small bowel. • Multiple paraaortic lymph nodes.
Recurrent sites confirmed by histopathology	Splenic tumor, Tumors involved mesocolon descendens and small bowel	Tumor involved mesocolon descendens	Massive tumor at left paracolic gutter, Tumors involved ileum and meso-ileum, Paraaortic lymph nodes.
Estimated blood loss	150ml	200ml	500ml

SCR, secondary cytoreduction; TCR, tertiary cytoreduction; QCR, quaternary cytoreduction; TFIp, treatment-free survival for platinum-based chemotherapy.

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Supplementary Table S2. Alterations of histologic features within multiple recurrence.

	SCR in first recurrence	TCR in second recurrence	QCR in third recurrence
Cytopathologic changes			
Markedly atypical cell	Focal	Dispersed	Easily to find
Mitosis/mm²	12	15	18
Histologic features of the stroma			
Architecture	Solid architecture predominant, with cribriform and papillary architecture	Papillary architecture	Papillary architecture
Neovascularization	Rare	Abundant with enlarged vessel	Angiogenic burst with massive, enlarged vessel
Hemorrhage	Partially disseminated hemorrhage	Disseminated hemorrhage	Massive hemorrhage
Necrosis	Rare	Partially frequent	Partially frequent
Mesenchyma	Less	Less	Less
Cancer-associated fibroblast	Rare	Rare	Rare[1]
Immunostaining			
CD8	5%	1%	<1 %
CD34	Few neovascularization	Intermediate neovascularization	Abundant neovascularization
CD31	Few neovascularization	Intermediate neovascularization	Abundant neovascularization

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PD-L1	CPS 40	CPS 20	CPS 5
Ki-67	Positive, 70%	Positive, 90%	Positive, 90%
p53	No expression	No expression	No expression

SCR, secondary cytoreduction; TCR, tertiary cytoreduction; QCR, quaternary cytoreduction; CPS, combined positive score.
[1] showed a significantly false positive staining in vessels.