Do increased tumor infiltrating lymphocytes co-existing with Homologous Recombination Deficiency provide clues to enhance immunotherapy of ovarian cancer?

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Not a week goes by without new publications on the benefit of immunotherapy to treat cancer, yet for ovarian cancer, a disease that continues to have a high mortality, the results of immunotherapy have so far been disappointing. The initial preliminary report of the effect of the PD1 Inhibitor, nivolumab in ovarian cancer was encouraging [1] but it has been followed by a large number of phase I/II trials, and more recently randomised phase III trials, some presented, but largely unpublished - with disappointing results. Why is this so? To understand this better, we need to study the biology of ovarian cancer more closely, not only to identify biomarkers for response but to look at the interaction of different biological processes within the tumor micro-environment. There are good reasons to believe that at least some ovarian cancers may benefit from immunotherapy and the design of future trials needs to take account of factors likely to lead to a more positive outcome. The presence of tumor-infiltrating lymphocytes (TILs) has consistently been seen to confer a better prognosis [2] but there are complex interactions within the tumor microenvironment among cells and molecules that exert a negative effect, such as tumor-associated macrophages (TAMs), CTLA4, LAG3, TIM3 etc[3]. A delicate interaction of these factors may make a major contribution to the presence of absence of an immunologic effect on the tumor.

Clinical observations indicate that ovarian cancer patients with a BRCA mutation have a higher survival rate, at least over the first few years, and tumors in these patients have been found to have higher numbers of immune cell infiltrates [4]. Survival differences conferred by BRCA mutations were known before the advent of PARP inhibitors, suggesting that there may be some relationship between immunity and BRCA deficiency. The hallmark of BRCA mutations is impaired repair of damaged DNA through a deficiency in homologous recombination (HRD). This is

now known to be more widespread in high grade ovarian cancer, affecting about half of all tumors. Mutations in several other genes in the HR pathway and hypermethylation of the BRCA1 promoter may all contribute to the phenotype of HRD. Whether the association of BRCA1/2 mutations and an increase in TILs extends to a broader group of HRD positive tumors has remained an open question. This has been addressed in the study reported *Morse and colleagues [Ref].* Do tumors with HRD have an increase in immune cell infiltrates, and does an association of these factors confer a better outcome? The group from University of Washington studied 250 consecutive tumor bank samples taken from patients undergoing primary surgery for ovarian cancer. HRD testing using BROCA sequencing identified cases with a mutation in one of 12 HRR genes or promoter methylation of BRCA1 or RAD51C[5]. HRD and non-HRD cases were well balanced with respect to known common prognostic factors such as stage, grade and histology although optimal cytoreduction was seen in more of the HRD cases. Survival was superior in HRD cases. An independent immunohistochemistry scoring mechanism for CD3+ TIL cells and CD68 tissue-associated macrophages was applied and a survival analysis demonstrated an improved survival for HRD+ cases as well as CD3+ TIL high cases. Multivariate analysis demonstrated that patients with HRD + and high CD3+ TIL scores had the best outcome with a median overall survival of a 70.9 months in HRD positive and CD3+ high cases and 35.8 months in non-HRD CD3+ low. There was no adverse effect on outcome seen with different levels of TAMs, as measured by CD68+ scores. This study supports the findings of smaller studies demonstrating an association between BRCA mutation, TILs and outcome [4] but the current study is larger, broadens the testing to include HRD and

has demonstrated independent effects of both HRD and CD3+ infiltration on outcome.

Two key questions arise from these findings. Firstly, is there a causal mechanism between HRD and CD3+TILs that leads to an improved outcome, and secondly can drugs that increase DNA damage stress response, such as PARP inhibitors further increase HRD and improve the immune microenvironment facilitating immunotherapeutic targeting of tumors? It has been hypothesised that the best results of immunotherapy are likely to occur in tumors with a high mutational burden [6] and a more favorable prognosis is seen patients with BRCA1/2 tumors with a higher neoantigen load, and this correlates with the number of CD3+ and CD8+ TILs and PD-1/PDL-1 expression[7]. The second issue, a mechanistic link between PARP inhibitors, the presence of HRD and immunogenicity is harder to conceptualise. However, there is growing evidence that the action of PARP inhibitors in HRD tumors leads to activation of the STING pathway, immune stimulation and increase in PD1/PD-1 expression[8, 9].

Clinical studies combining inhibitors of PARP and PD1/PDL-1 are now being tested widely in the clinic. Preliminary non-randomised data from the TOPACIO trial (niraparib/pembrolizumab) [10] and the combination of durvalumab and olaparib [11] have demonstrated higher response rates than one might have expected from either drug alone in the population studied. The pharmaceutical industry in particular is anticipating that this combination will lead to a better outcome than using immune checkpoint inhibitors alone and have moved rapidly to conducting randomised studies in the first line setting. The stakes are high, and one of the five trials has already been abandoned shortly after starting, based on poor results of an immune

checkpoint inhibitor alone in first-line therapy. Neither this trial nor the current first-line studies pay attention to the tumour micro-environment. It is here that we will learn more about which patients will or will not benefit from immunotherapy. PDL1 expression is just one factor; CD3+, CD8+ TIL is another but the interaction of these with inhibitory factors, such as tissue macrophages is still not well understood.

References

[1] Hamanishi J, Mandai M, Ikeda T, Minami M, Kawaguchi A, Murayama T, et al. Safety and Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33:4015-22.

[2] Hwang WT, Adams SF, Tahirovic E, Hagemann IS, Coukos G. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. Gynecologic oncology. 2012;124:192-8.

[3] Barnes TA, Amir E. HYPE or HOPE: the prognostic value of infiltrating immune cells in cancer. British journal of cancer. 2017;117:451-60.

[4] McAlpine JN, Porter H, Kobel M, Nelson BH, Prentice LM, Kalloger SE, et al. BRCA1 and BRCA2 mutations correlate with TP53 abnormalities and presence of immune cell infiltrates in ovarian high-grade serous carcinoma. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2012;25:740-50.

[5] Pennington KP, Walsh T, Harrell MI, Lee MK, Pennil CC, Rendi MH, et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20:764-75.

[6] Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013;500:415-21.

[7] Strickland KC, Howitt BE, Shukla SA, Rodig S, Ritterhouse LL, Liu JF, et al. Association and prognostic significance of BRCA1/2-mutation status with neoantigen load, number of tumor-

infiltrating lymphocytes and expression of PD-1/PD-L1 in high grade serous ovarian cancer. Oncotarget. 2016;7:13587-98.

[8] Ding L, Kim HJ, Wang Q, Kearns M, Jiang T, Ohlson CE, et al. PARP Inhibition Elicits STING-Dependent Antitumor Immunity in Brca1-Deficient Ovarian Cancer. Cell Rep. 2018;25:2972-80 e5.

[9] Jiao S, Xia W, Yamaguchi H, Wei Y, Chen MK, Hsu JM, et al. PARP Inhibitor Upregulates PD-L1 Expression and Enhances Cancer-Associated Immunosuppression. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017;23:3711-20.

[10] Konstantinopoulos PA, Waggoner SE, Vidal GA, Mita MM, Fleming GF, Holloway RW, et al. TOPACIO/Keynote-162 (NCT02657889): A phase 1/2 study of niraparib + pembrolizumab in patients (pts) with advanced triple-negative breast cancer or recurrent ovarian cancer (ROC)—Results from ROC cohort. Journal of Clinical Oncology. 2018;36:106-.

[11] Lee JM, Cimino-Mathews A, Peer CJ, Zimmer A, Lipkowitz S, Annunziata CM, et al. Safety and Clinical Activity of the Programmed Death-Ligand 1 Inhibitor Durvalumab in Combination With Poly (ADP-Ribose) Polymerase Inhibitor Olaparib or Vascular Endothelial Growth Factor Receptor 1-3 Inhibitor Cediranib in Women's Cancers: A Dose-Escalation, Phase I Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2017;35:2193-202.