Definitive study shows no association between ARID1A mutation status and clinical outcome in endometriosis-related ovarian cancers

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‡Invited commentary for K Heinze, TM Nazeran et al. Validated biomarker assays confirm that ARID1A loss is confounded with MMR deficiency, CD8+ TIL infiltration, and provides no independent prognostic value in endometriosis-associated ovarian carcinomas. J Pathol 2022; 256: 388–401.

Abstract

The ARID1A tumour suppressor protein is a component of the SWI/SNF chromatin remodelling complex, which is mutated in approximately 20% of all human cancers. ARID1A mutational status is considered to hold prognostic significance in a range of solid malignancies, yet in endometriosis-related ovarian carcinomas there has been a lack of clarity of its prognostic role. Moreover, the relationship between ARID1A status and immune infiltrate is also poorly understood. In a recent issue of The Journal of Pathology, a large comprehensive study by Heinze, Nazeran et al addressed these areas by reviewing 1,623 endometriosis-associated ovarian carcinomas and correlating ARID1A status using standardised immunohistochemistry to infer mutation status, with comprehensive clinicopathological features, mismatch repair status and CD8+ tumour infiltrating lymphocytes. The study definitively showed that ARID1A status does not provide any independent prognostic value in endometriosis-associated ovarian carcinomas. ARID1A loss was, however, shown to be associated with mismatch repair deficiency and increased CD8+ tumour infiltrating lymphocytes in endometrioid ovarian carcinoma, which may be relevant for future studies.

Keywords: ARID1A; CD8 tumour infiltrating lymphocytes; mismatch repair deficiency; biomarker; endometriosis-associated ovarian carcinomas

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ovarian cancer cases \( (n = 2,548) \). Importantly, the authors showed that ARID1A loss is not an independent prognostic factor for either ENOC or CCOC when assessing a variety of survival metrics, including overall survival, progression-free survival and disease-specific survival. They confirmed, however, the importance of prognostic factors, such as the presence of residual disease and stage, with residual disease and higher stage being associated with poorer outcomes.

A smaller cohort of tumours was assessed for CD8+ TILs within the tumour epithelium (933 ENOC and 480 CCOC), with ARID1A loss being associated with statistically significant higher CD8+ TILs in ENOC but not in CCOC, although a trend to higher CD8+ TILs was seen. A modest yet statistically significant overall survival benefit was observed in ENOC patients with high CD8+ TILs scores. No such trend was observed in CCOC. CD8+ TILs were significantly associated with MMR deficiency (MMRd) in ENOC and CCOC tumours, with an overall rate of MMRd of 13% of ENOC tumours and 5% of CCOC tumours. A significant association between ARID1A loss and MMRd was noted, present in 22% of all ENOC, highlighting that ARID1A loss is probably confounded by MMRd, given MMRd results in a high mutational burden in an otherwise genomically quiet tumour type.

We would like to commend the authors on this impressive large-scale international effort highlighting that ARID1A IHC is a reproducible and reliable test for loss of function mutational status, which can be used in mainstream pathology laboratories. Moreover, the authors provide clarity and definitive evidence of the lack of prognostic significance of ARID1A in EAOC. However, these results do not discount the importance of developing therapeutic strategies to target tumours with loss of ARID1A and the clinical relevance of investigating the possible predictive value of ARID1A for immune-modulatory therapies, given the overall poor clinical outcome in these disease types. One approach utilising synthetic-lethal approaches for targeting ARID1A-deficient cancers with the ATR inhibitor cerlatertib with or without the PARP (poly ADP ribose polymerase) inhibitor olaparib is being assessed in the ATARI phase II international proof-of-concept clinical trial. In this trial, ARID1A IHC is being used upfront to stratify patients with ovarian and endometrial clear cell carcinoma, together with other rare gynaecological tumours, into the two treatment arms depending on their ARID1A status.

In this current study, the authors used tissue microarrays as a practical method for obtaining an initial insight into the pathology of each sample. One limitation is that a tissue microarray may fail to accurately capture subclonal staining patterns and intra-tumoural heterogeneity. In addition, the logistics of sequencing large numbers of patient samples to assess tumour mutational burden and to determine POLE (DNA polymerase \( \varepsilon \) ) status was not possible. Future studies incorporating these elements and serial patient samples would allow a deeper assessment with respect to modern molecular prognostic subtypes of ENOC, tumour evolution and responses to treatment.

Overall, the authors showed that loss of ARID1A is associated with higher CD8+ TILs in ENOC and intratumoural CD8+ immune cells suggestive of a possible role for immunotherapy. Low response rates have been demonstrated in initial clinical trials in relapsed ovarian cancer. However, CCOC has shown improved response rates compared with other epithelial ovarian cancers, including the highest response rate of 15.8% to pembrolizumab in the phase II KEYNOTE-100 trial, compared with 8.5% in unselected ovarian cancer. NINJA, a randomised phase III trial in platinum-resistant ovarian cancer patients, assessed nivolumab compared with chemotherapy, showing no statistically significant survival benefit, but a numerically longer overall survival.

Table 1. Relevant immunotherapy trials in clear cell gynaecological cancers.

| Study title | Phase | Treatment | Primary aims | Secondary aims | Patients (n) | Molecular target | Identifier, Status |
|-------------|-------|-----------|--------------|----------------|-------------|-----------------|-------------------|
| BRUOG 354 | II | Nivolumab ± ipilimumab for ovarian and extra-renal clear cell carcinomas | PFS | PFS | 62 | PD-1 and CTLA4 | NCT03355976 Recruiting |
| Nivolumab and ipilimumab in treating patients with rare tumours | II | Nivolumab and ipilimumab | ORR | AE, BOR, CBR, DO, PFS | 707 | PD-1 and CTLA4 | NCT02834013 Recruiting |
| A multicentre phase II trial of durvalumab versus physician’s choice chemotherapy in recurrent ovarian clear cell adenocarcinomas (MOCCA) | II | Durvalumab versus standard cytotoxic chemotherapy | PFS | ORR, OS, AE, QOL | 46 | PD-L1 | NCT0405454 Results awaited |
| A phase II study of pembrolizumab in patients with advanced gynaecological clear cell cancer (PEACOCC) | II | Pembrolizumab | PFS | QOL | 48 | PD-1 | NCT03425565 active not recruiting. Results awaited NCT04699071 Recruiting |
| Phase II trial of lenvatinib plus pembrolizumab in recurrent gynecological clear cell adenocarcinomas (LARA) | II | Lenvatinib and pembrolizumab | ORR | PFS, DOR | 10 | RTK (VEGFR1, VEGFR2, VEGFR3) and PD-1 | NCT04699071 Recruiting |

AE, adverse event; BOR, best overall response; CBR, clinical benefit rate; DOR, duration of response; OS, overall survival; ORR, overall response rate; PFS, progression-free survival; QOL, quality of life; RTK, receptor tyrosine kinase.
in CCOC patients (Table 1 highlights ongoing relevant immunotherapy-based trials). Although Heinze and colleagues show a significant association between ARID1A loss and MMRd, the clinical impact of MMR status in the context of ARID1A loss is not understood and the translational research from the above studies will help to further elucidate the relevance of ARID1A loss depending on MMR status and response to immunotherapy.

Although the authors scored CD8+ cells, further key immune subpopulations and their spatial locations were not characterised and correlated with ARID1A mutational status and clinicopathological features. The CD8+ TILs scoring methodology was a semiquantitative approach that only considered CD8+ TILs within the epithelial component of the tumour, disregarding stromal CD8+ cells [2]. Recently, Khalique et al [4] quantified and assessed the spatial locations of various immune subpopulations in 31 microsatellite stable CCOCs using multiplexed immunofluorescence. ARID1A mutant cases showed significantly higher CD8+ cells and CD68+ cells (tumour-associated macrophages, TAMs) in the stroma relative to tumour. The spatial location of these immune subpopulations has also been shown to hold clinical significance in a range of solid malignancies, such as colorectal, breast and lung cancer [5–8]. In squamous lung cell cancer, for example, stromal TAMs have been shown to interact with stromal CD8+ cells, and this interaction results in a reduction in motility of CD8+ cells in the tumour microenvironment, ‘trapping’ them in the stroma and contributing to a T-cell excluded tumour phenotype associated with worse outcome [9]. This interaction would suggest that targeting TAMs could be relevant in synergising immune checkpoint-based immunotherapy in various tumour types, and in the context of endometriosis-related ovarian cancers, specifically ARID1A-mutated tumours. Khalique et al [4] also found significantly higher numbers of immunosuppressive subpopulations (TAMs and FOXP3+/CD4+ regulatory T-cells) in the stroma relative to tumours of patients with improved outcomes, suggesting that the ‘tumour-exclusion’ of these cells is important in maintaining an effective anti-tumour immune response. It would be useful to validate these spatial findings in larger cohorts, such as that of Heinze, Nazeran et al [2].

Recent studies have additionally used gene expression profiling to identify prognostic gene expression signatures in CCOCs. Tan et al [10] identified two prognostic CCOC gene expression subtypes, including a mesenchymal subtype characterised by a high immune/inflammatory gene expression profile but not driven by CD8+-specific signatures. Khalique et al [4] also identified and validated an immune-related gene expression signature associated with clinical outcome in CCOC. Gene expression analysis in the large cohort of Heinze, Nazeran et al [2] would provide valuable additional data to prognosticate these tumours.

This large current study highlights the importance of maintaining clinical databases (although this study was comprised of predominantly Caucasian patients), with international registry collaborations enabling a robust and powerful dataset with which one can draw definitive conclusions. ARID1A has been shown not to be an independent prognostic biomarker and is confounded by MMRd in EAOC. Ongoing research is needed to determine its clinical relevance in additional ethnic populations in a rapidly evolving landscape of treatment options and to take these findings into consideration when interpreting the results of trials where known biomarkers of response include MMRd.

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Author contributions statement

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References

1. Shen J, Ju Z, Zhao W, et al. ARID1A deficiency promotes mutability and potentiates therapeutic antitumor immunity unleashed by immune checkpoint blockade. Nat Med 2018; 24: 556–562.
2. Heinze K, Nazeran TM, Lee S, et al. Validated biomarker assays confirm that ARID1A loss is confounded with MMR deficiency, CD8+ TIL infiltration, and provides no independent prognostic value in endometriosis-associated ovarian carcinomas. J Pathol 2022; 256: 388–401.
3. Khalique S, Naidoo K, Attygalle AD, et al. Optimised ARID1A immunohistochemistry is an accurate predictor of ARID1A mutational status in gynaecological cancers. J Pathol Clin Res 2018; 4: 154–166.
4. Khalique S, Nash S, Mansfield D, et al. Quantitative assessment and prognostic associations of the immune landscape in ovarian clear cell carcinoma. Cancers (Basel) 2021; 13: 3854.
5. Zheng X, Weigert A, Reu S, et al. Spatial density and distribution of tumour-associated macrophages predict survival in non-small cell lung carcinoma. Cancer Res 2020; 80: 4414–4425.
6. Gruosso T, Gigoux M, Manem VSK, et al. Spatially distinct tumor immune microenvironments stratify triple-negative breast cancers. J Clin Invest 2019; 129: 1785–1800.
7. Zwing N, Failmezger H, Ooi CH, et al. Analysis of spatial organization of suppressive myeloid cells and effector T cells in colorectal cancer—a potential tool for discovering prognostic biomarkers in clinical research. Front Immunol 2020; 11: 550250.
8. Nearchou IP, Gwyther BM, Georgiakakis ECT, et al. Spatial immune profiling of the colorectal tumor microenvironment predicts good outcome in stage II patients. NPJ Digit Med 2020; 3: 71.
9. Peranzoni E, Lemoine J, Vimeux L, et al. Macrophages impede CD8 T cells from reaching tumor cells and limit the efficacy of anti-PD-1 treatment. Proc Natl Acad Sci U S A 2018; 115: E4041–E4050.
10. Tan TZ, Ye J, Yee CV, et al. Analysis of gene expression signatures identifies prognostic and functionally distinct ovarian clear cell carcinoma subtypes. ElBioMedicine 2019; 50: 203–210.