ARID1A Immunohistochemistry (IHC) Shows Low Sensitivity for *ARID1A* Mutation by Next Generation Sequencing (NGS) in Endometrial Carcinomas

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Background

ARID1A mutations (*ARID1A*mut) are a potential marker of poor prognosis in endometrial carcinomas (EC). Ongoing development of novel therapies targeting *ARID1A*mut tumors further emphasizes the importance of accurately identifying these cases.

Purpose:

- Define the concordance of ARID1A IHC with *ARID1A* mutation status by NGS.
- Identify patterns of discordance.
- Evaluate tumors with heterogeneous ARID1A IHC (hetARID1A) expression by performing differential microdissection (MD) and NGS.

Design

With IRB exemption, EC resections at a single tertiary care center from 2022-2023 were evaluated (n = 146).

All cases underwent TCGA/ProMisE classification

- *POLE* by NGS
- MMR and p53 by IHC

All cases underwent ARID1A IHC (EP303, CellMarque):

- retARID1A (retained ARID1A expression)
- lostARID1A (lost ARID1A expression)
- hetARID1A (discrete areas of both retention and loss)

NGS included *ARID1A* assessment in 107 cases

4 hetARID1A cases underwent MD and NGS analysis

Pearson Chi-square statistics ($p < 0.05$)

ARID1A NGS Status	ARID1A IHC Status			
	Hetero	Lost	Retained	Total
POLE				
ARID1A Mutant	0	0	1	1
ARID1A WT	0	0	3	3
MMRd				
ARID1A Mutant	2	10	7	19
ARID1A WT	2	0	8	10
NSMP				
ARID1A Mutant	7	7	14	31*
ARID1A WT	0	0	21	21
p53abn				
ARID1A Mutant	0	0	3	3
ARID1A WT	0	0	22	22
Total	11	17	79	107

Table 1: *ARID1A*mut status as a function of ARID1A IHC status and TCGA/ProMisE classification. *$p < 0.05$

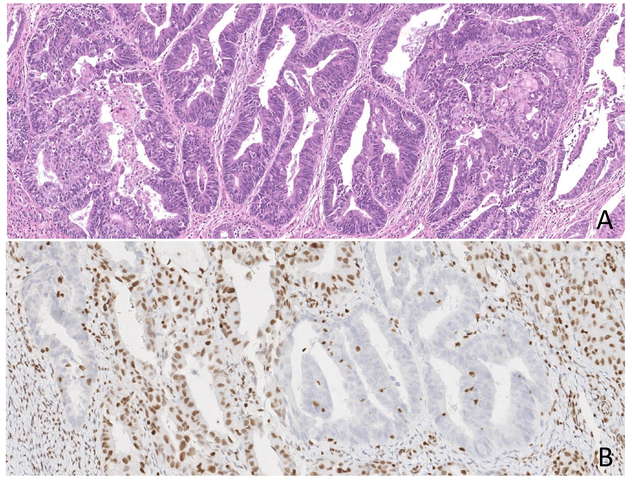

Figure 1: Representative case of hetARID1A. A) H&E, 100X; B) ARID1A IHC, 100X; areas of heterogenous expression

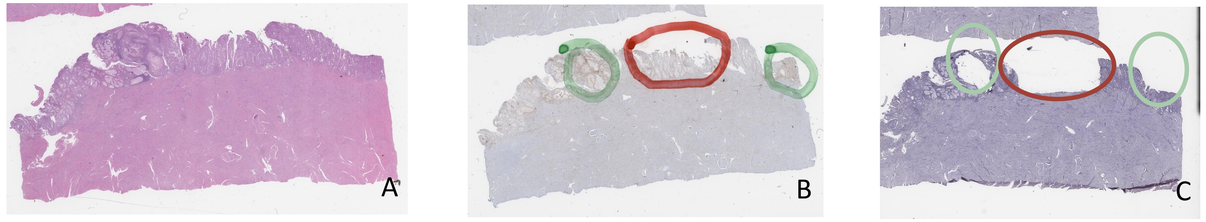

Figure 2: Representative hetARID1A microdissection. A) H&E, endomyometrium; B) ARID1A IHC, retARID1A circled green, lostARID1A circled red; C) Hematoxylin; post-MD; retARID1A circled green, lostARID1A circled red

Results

Of 107 EC tested, 51 (48%) were *ARID1A*mut by NGS

- 25/79 (32%) retARID1A IHC cases were *ARID1A*mut
- 9/11 (82%) hetARID1A IHC cases were *ARID1A*mut

IHC (het/lost) detected *ARID1A*mut with 51% sensitivity and 96% specificity

All 17 cases with lostARID1A had >1 *ARID1A* mutation

Of 34 *ARID1A*mut cases with retARID1A or hetARID1A, 14 (41%) had evidence of double-hit in *ARID1A*

- 4 with copy neutral loss of heterozygosity (CNLOH)
- 10 with biallelic mutation
- 8 with sub-clonality

NSMP enriched for *ARID1A*mut ($p < 0.05$)

- *ARID1A*mut differed by ProMisE classification (Table 1)

4/11 cases with hetARID1A underwent MD/NGS

- *ARID1A*mut were seen in areas of both retARID1A and lostARID1A for all 4 cases
- 2 had divergent tumor clonality
- 1 had clonal selection

Conclusions

- In EC, ARID1A IHC (EP303) shows low sensitivity (51%) for *ARID1A*mut, suggesting its inadequacy as a singular screening modality for *ARID1A* status.
- Reasons for discordant IHC and NGS are yet to be elucidated, and may relate to antibody epitope location, cross-reactivity, or epigenetic changes, among others.
- Accurate assessment of *ARID1A*mut status is likely to require molecular characterization.

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