AWARD NUMBER: W81XWH-22-1-0156

TITLE: Therapeutic Targeting of Mevalonate Pathway in ARID1A-Mutated Ovarian Cancer

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CONTRACTING ORGANIZATION: The Wistar Institute**,** Philadelphia, PA

REPORT DATE: October 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2023	Annual	01Sep2022-31Aug2023

4. TITLE AND SUBTITLE

Therapeutic Targeting of Mevalonate Pathway in ARID1A-Mutated Ovarian Cancer

5a. CONTRACT NUMBER
W81XWH-22-1-0156

5b. GRANT NUMBER
OC210124

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Wistar Institute
3601 Spruce Street
Philadelphia, PA 19104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

ARID1A encodes a subunit of the SWI/SNF chromatin-remodeling complex and functions as a tumor suppressor. Notably, inactivating mutations in *ARID1A* occur frequently in ovarian clear cell carcinomas (OCCC; >50%) and ovarian endometrioid carcinomas (OEC; >30%). There is an unmet need for effective treatment modalities for *ARID1A*-mutated ovarian cancers. Emerging evidence supports the idea that the SWI/SNF complexes play a critical role in the tumor metabolism. Our preliminary data show that *ARID1A* mutation suppresses the mevalonate pathway through downregulating rate-limiting enzymes such as 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) and HMG-CoA reductase (HMGCR). The mevalonate pathway has previously been implicated in cancers due to its essential role in cell survival and proliferation. Indeed, ARID1A inactivation sensitizes ovarian cancer to the inhibition of residual mevalonate pathway activity using FDA-approved, clinically applicable inhibitors such as Simvastatin and Atorvastatin. Pyroptosis is a lytic nonapoptotic cell death that triggers an inflammatory response. Our preliminary data suggest that statins induce pyroptosis in an ARID1A status dependent manner. In addition, our preliminary data show that *ARID1A* mutation sensitizes ovarian cancer to immune checkpoint blockades such as anti-PD-L1 treatment. Notably, pyroptosis induction in cancer cells promotes infiltration of immune cells such as $CD8^+$ effective T cells that is required for the response to immune checkpoint blockades. Together, these findings raised the possibility that the inhibition of the mevalonate pathway by statins in *ARID1A*-mutated ovarian cancer will not only suppress the growth of *ARID1A* mutant cancer cells but also promote the infiltration of immune cells such as $CD8^+$ effective T cells and thus enhance immune checkpoint blockade therapy. Our **central hypothesis** is that *ARID1A*-mutated ovarian cancer can be therapeutically eradicated by a combination of FDA-approved mevalonate pathway inhibitors such as statins and anti-PD-L1 immune checkpoint blockade.

15. SUBJECT TERMS
Epithelial ovarian cancer, ovarian clear cell carcinoma, ARID1A, SWI/SNF, mevalonate pathway, immune checkpoint blockade

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE	Unclassified	11	USAMRDC
Unclassified	Unclassified	Unclassified			19b. TELEPHONE NUMBER (include area code)

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. INTRODUCTION:

ARID1A encodes a subunit of the SWI/SNF chromatin-remodeling complex and functions as a tumor suppressor. Notably, inactivating mutations in *ARID1A* occur frequently in ovarian clear cell carcinomas (OCCC; >50%) and ovarian endometrioid carcinomas (OEC; >30%). There is an unmet need for effective treatment modalities for *ARID1A*-mutated ovarian cancers. Emerging evidence supports the idea that the SWI/SNF complexes play a critical role in the tumor metabolism. Our preliminary data show that *ARID1A* mutation suppresses the mevalonate pathway through downregulating rate-limiting enzymes such as 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) and HMG-CoA reductase (HMGCR). The mevalonate pathway has previously been implicated in cancers due to its essential role in cell survival and proliferation. Indeed, ARID1A inactivation sensitizes ovarian cancer to the inhibition of residual mevalonate pathway activity using FDA-approved, clinically applicable inhibitors such as Simvastatin and Atorvastatin. Pyroptosis is a lytic nonapoptotic cell death that triggers an inflammatory response. Our preliminary data suggest that statins induce pyroptosis in an ARID1A status dependent manner. In addition, our preliminary data show that *ARID1A* mutation sensitizes ovarian cancer to immune checkpoint blockades such as anti-PD-L1 treatment. Notably, pyroptosis induction in cancer cells promotes infiltration of immune cells such as $CD8^+$ effective T cells that is required for the response to immune checkpoint blockades. Together, these findings raised the possibility that the inhibition of the mevalonate pathway by statins in *ARID1A*-mutated ovarian cancer will not only suppress the growth of *ARID1A* mutant cancer cells but also promote the infiltration of immune cells such as $CD8^+$ effective T cells and thus enhance immune checkpoint blockade therapy. Our **central hypothesis** is that *ARID1A*-mutated ovarian cancer can be therapeutically eradicated by a combination of FDA-approved mevalonate pathway inhibitors such as statins and anti-PD-L1 immune checkpoint blockade.

KEYWORDS:
Epithelial ovarian cancer, ovarian clear cell carcinoma, ARID1A, SWI/SNF, mevalonate pathway, immune checkpoint blockade

2. ACCOMPLISHMENTS:

What were the major goals of the project?

The objective of this proposal is this application is to develop a novel therapeutic strategy for ARID1A-mutated ovarian cancers by combining clinically applicable mevalonate pathway inhibitors such as simvastatin and immune checkpoint anti-PD-L1.

Accordingly, **two specific aims** are proposed:

Specific Aim 1: To investigate the mechanism underlying the dependence of *ARID1A* mutant ovarian cancer cells on the mevalonate pathway.
Specific Aim 2: To develop a novel therapeutic approach for *ARID1A*-mutated ovarian cancer by combining FDA-approved mevalonate pathway inhibitor statins and anti-PD-L1.

What was accomplished under these goals?

Since the starting of the award, substantial progress has been made toward achieving the goals as outlined in the application.

1) major activities;
The major activities in the first year are as following:

1. We established the condidation and performed ARID1A ChIP analysis on the HMGCS1 and HMGCR gene promoters.
2. We established the condition and perform ChIP analysis for RNA polymerase II and other subunits of the SWI/SNF complex on the *HMGCS1* and *HMGCR* gene promoters in ARID1A wildtype OCCC cell lines with or without ARID1A knockout.

3. We correlated changes in the association of ARID1A, RNA Pol II and other subunits of the SWI/SNF with the *HMGCS1* and *HMGCR* promoters with changes in HMGCS1 and HMGCR expression.
4. We correlated correlate *ARID1A* mutational status with response to inhibition of the mevalonate pathway by FDA-approved statins.

2) specific objectives;
The major objectives in the first year of funding are as following:

1. To establish the condidation and performed ARID1A and RNA polymerase II ChIP analysis on the HMGCS1 and HMGCR gene promoters.
2. To correlate changes in the association of ARID1A, RNA Pol II and other subunits of the SWI/SNF with the *HMGCS1* and *HMGCR* promoters with changes in HMGCS1 and HMGCR expression..
3. To correlate *ARID1A* mutational status with response to inhibition of the mevalonate pathway by FDA-approved simvastatins.

3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative);

***HMGCR* and *HMGCS1* are direct target genes of the ARID1A-containing SWI/SNF complex**
We next validated the association of ARID1A and SNF5, a core subunit of the SWI/SNF complex [1], with the promoters of the *HMGCR* and *HMGCS1* genes (**Fig. 1A-B**). *ARID1A* knockout reduced the association of SNF5 and RNA Pol II with their promoters (**Fig. 1A-B**). Conversely, wildtype ARID1A restoration in *ARID1A*-mutated TOV21G cells enhanced the association of both SNF5 and RNA Pol II with the promoters of the *HMGCR* and *HMGCS1* genes (**Fig. 1C-D**). This data supports that the ARID1A-containing SWI/SNF complex functions as an activator of *HMGCR* and *HMGCS1* expression.

We next validated the downregulation of both HMGCR and HMGCS1 at protein levels by *ARID1A* knockout in RMG1 and OVCA429 cells (**Fig. 2A-B**). Ectopic wildtype ARID1A restoration rescued the downregulation of HMGCR and HMGCS1 induced by *ARID1A* knockout (**Fig. 2A-B**). Likewise, ectopic wildtype ARID1A restoration in *ARID1A*-mutated OCCC cell lines such as TOV21G and OVISE upregulated both HMGCS1 and HMGCR (**Fig. 2C-D**). Thus, changes in the association of ARID1A, SNF5 and RNA Pol II with the HMGCS1 and HMGCR promoters correlated with changes in HMGCS1 nd HMGCR expression.

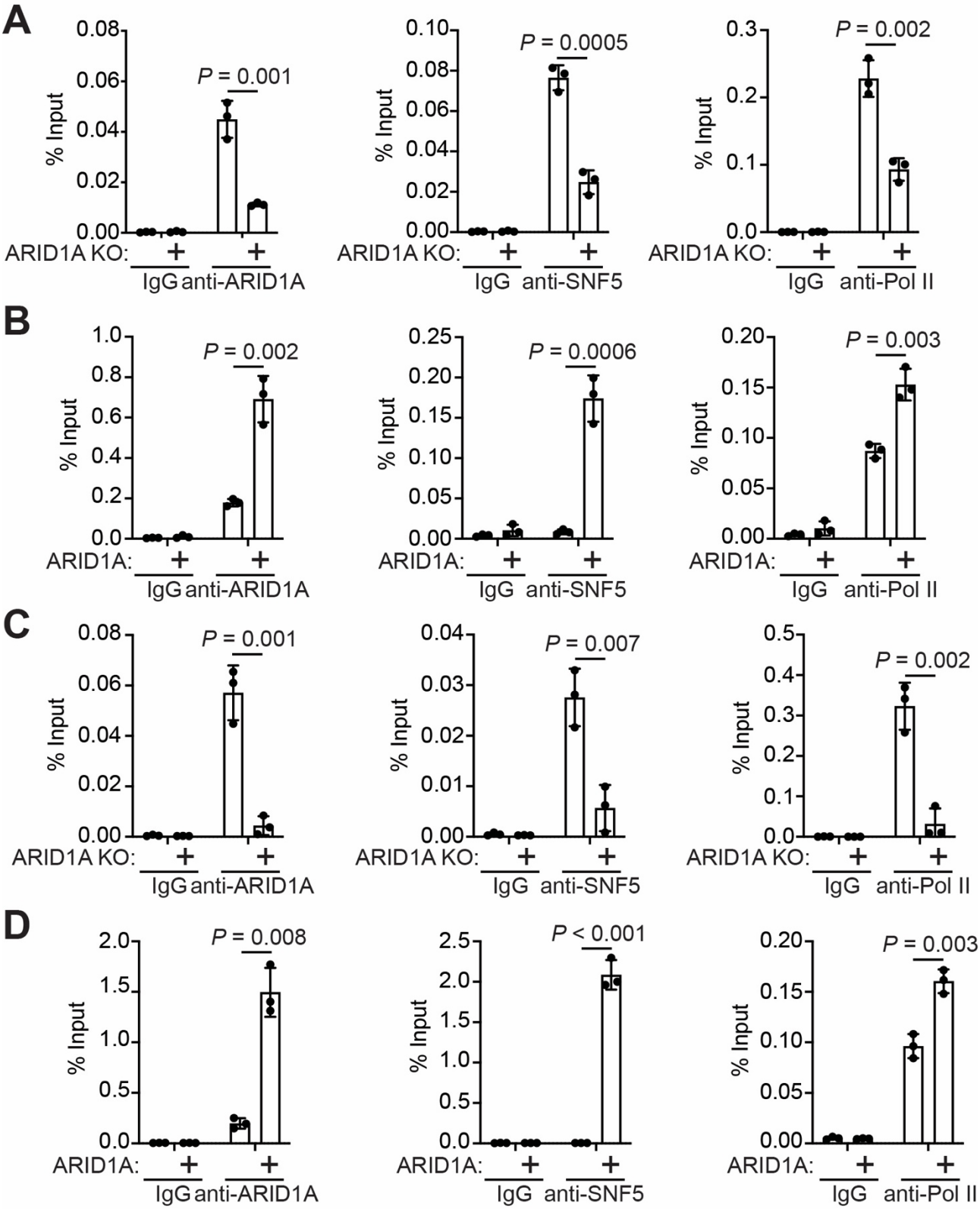

Figure 1: *HMGCS1* and *HMGCR* are direct target genes of the ARID1A-containing SWI/SNF-complex

A-B, Expression of the indicated proteins in control, *ARID1A* knockout RMG1 (A) or OVCA429 (B) cells with or without wildtype ARID1A restoration was determined by immunoblot.
C-D, Expression of the indicated proteins in control and wildtype ARID1A restored TOV21G (C) or OVISE (D) cells were determined by immunoblot.
Error bars represent mean with SEM. *P* values were calculated using two-tailed Student t-test.

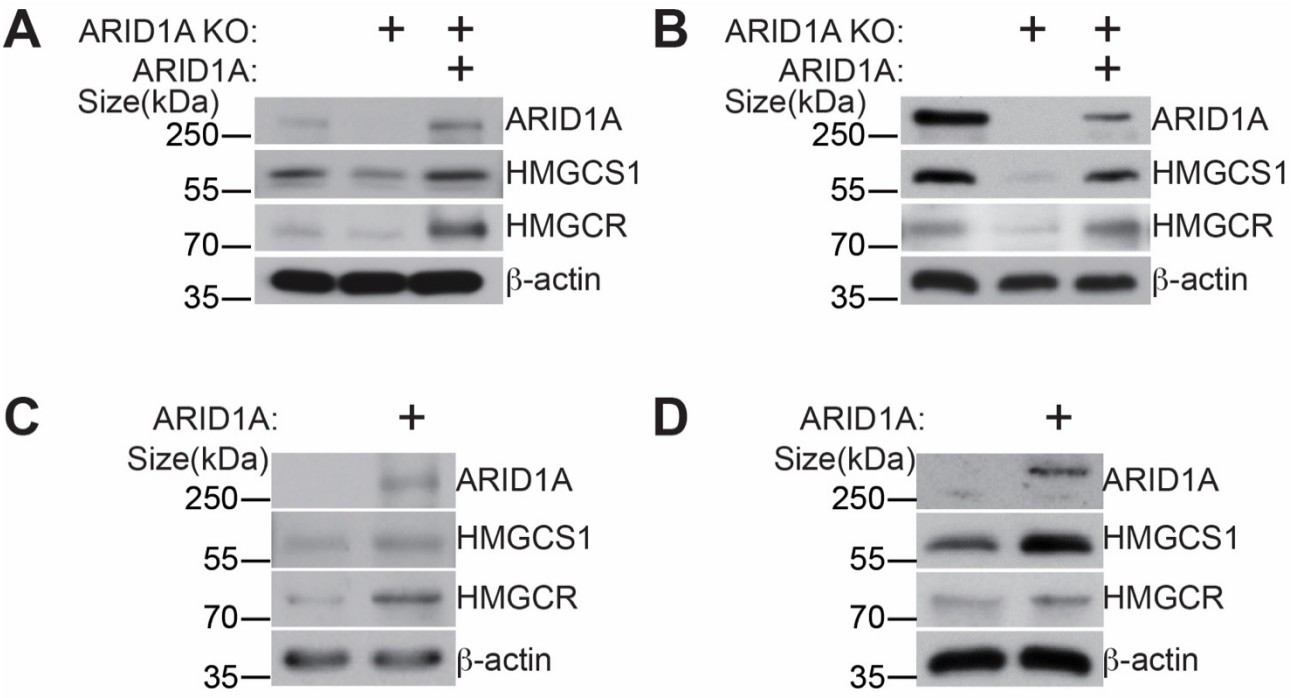

Figure 2: Expression of *HMGCS1* and *HMGCR* correlate with ARID1A status
The association of ARID1A, SNF5 and RNA Pol II with the *HMGCS1* and *HMGCR* gene promoters in parental and *ARID1A* knockdown RMG1 cells **(A** and **B)** or control and wildtype ARID1A restored TOV21G cells **(C** and **D)** was determined by ChIP-qPCR analysis. An isotype matched IgG was used as a control. n=3 independent experiments.
Error bars represent mean with SEM. *P* values were calculated using two-tailed Student t-test.

ARID1A status correlates with response to simvastatin
ARID1A knockout significantly reduced the IC_{50}s of simvastatin in *ARID1A* wildtype cells, which can be rescued by ectopic ARID1A expression (**Fig. 3A-B**). Conversely, wildtype ARID1A restoration in *ARID1A*-mutated OCCC cell lines such as TOV21G increased the IC_{50}s of statins (**Fig. 3C-D**). In a panel of OCCC cell lines, the IC_{50}s of simvastatin were significantly lower in *ARID1A* mutant compared with wildtype cell lines (**Fig. 3E-F**). Thus, we conclude that ARID1A status correlates with response to simvastatin.

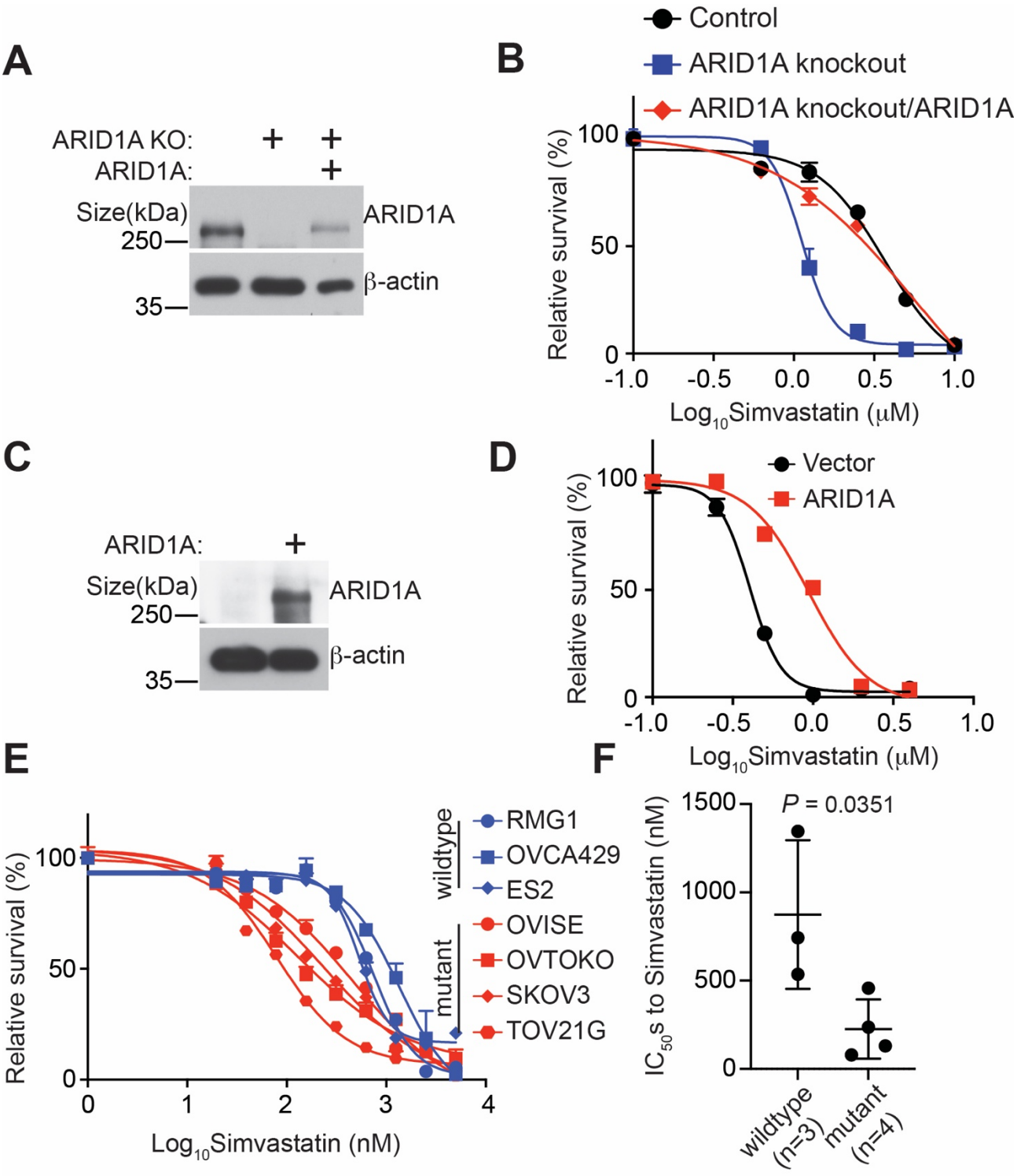

Figure 3: ARID1A status correlates with response to simvastatin

A, Expression of ARID1A in control and *ARID1A* knockout OVCA429 cells with or without wildtype ARID1A restoration was determined by immunoblot.

B, Dose response curves of the indicated control and *ARID1A* knockout OVCA429 cells with or without wildtype ARID1A restoration to simvastatin were determined by colony formation assay. n=4 independent experiments.

C-D, Expression of ARID1A in control and *ARID1A* mutant TOV21G cells with or without wildtype ARID1A restoration was determined by immunoblot (**C**). Dose response curves of the indicated control and wildtype ARID1A restored *ARID1A*-mutated TOV21G OCCC cells to simvastatin were determined by colony formation assay **(D)**. n=4 independent experiments.
E, Dose response curves of the indicated *ARID1A* wildtype or mutant OCCC cell lines to simvastatin were determined by the AlamarBlue assay. n=4 independent experiments.
F, IC_{50}s of simvastatin in *ARID1A* wildtype (RMG1, OVCA429 and ES2) and (OVISE, OVTOKO, SKOV3 and TOV21G) cell lines.
Error bars represent mean with SEM. *P* values were calculated using two-tailed Student t-test.

and/or 4) other achievements.

Nothing to report.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
We plan to do the following during the next reporting period to accomplish the goals:
1. To correlate *ARID1A* mutational status with response to inhibition of the mevalonate pathway genetically by knocking down the expression of rate-limiting enzymes such as HMGCS1 and HMGCR.
2. To determine whether HMGCS1/HMGCR status determines the response of ARID1A wildtype cells to inhibition of mevalonate pathway.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Nothing to report.

What was the impact on other disciplines?
Nothing to report.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Dr. Rugang Zhang, the PI, has moved his laboratory to The University of Texas MD Anderson Cancer Center on Feb. 1st 2023. The request to transfer the award witih Dr. Zhang has been submitted to CDMRP OCRP and we are waiting on the approval from OCRP to proceed with the transfer. This move will positively impact the proposed studies due to the accessibility to additional research cores.

Changes that had a significant impact on expenditures
Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report.

Significant changes in use or care of human subjects
Nothing to report.

Significant changes in use or care of vertebrate animals
Nothing to report.

Significant changes in use of biohazards and/or select agents
Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

1. Lombardi S, Goldman AR, Tang HY, Kossenkov AV, Liu H, Zhou W, Herlyn M, Lin J, **Zhang R.** Targeting Fatty Acid Reprogramming Suppresses CARM1-expressing Ovarian Cancer. **Cancer Res Commun**. 2023 Jun 20;3(6):1067-1077. doi: 10.1158/2767-9764.CRC-23-0030. eCollection 2023 Jun.PMID: 37377614 PMCID: PMC10281290

2. Zhou W, Liu H, Yuan Z, Zundell J, Towers M, Lin J, Lombardi S, Nie H, Murphy B, Yang T, Wang C, Liao L, Goldman AR, Kannan T, Kossenkov AV, Drapkin R, Montaner LJ, Claiborne DT, Zhang N, Wu S, **Zhang R.** Targeting the mevalonate pathway suppresses ARID1A-inactivated cancers by promoting pyroptosis. **Cancer Cell**. 2023 Apr 10;41(4):740-756.e10. doi: 10.1016/j.ccell.2023.03.002. Epub 2023 Mar 23.PMID: 36963401 PMCID: PMC10085864

Books or other non-periodical, one-time publications.
Nothing to report.

Other publications, conference papers and presentations.
Nothing to report.

Website(s) or other Internet site(s)
Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses
Nothing to report.

Other Products

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project? (09/01/22-01/31/23)

Name:	Rugang Zhang
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-7255-2360
Nearest person month worked:	.5
Contribution to Project:	Supervised the study.
Funding Support:	This award

Name:	Chen Wang
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	12
Contribution to Project:	Performed the study.
Funding Support:	This award

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? For the period 09/01/22-01/31/23.

Termination of OCRA596552 effective 12/31/21
Termination of W81XWH-19-1-0154 effective 09/29/22
Activation of 1R01CA260661-01A1 effective 08/01/22

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

N/A

9. APPENDICES:

1. Wu, J.N. and C.W. Roberts, *ARID1A mutations in cancer: another epigenetic tumor suppressor?* Cancer Discov, 2013. **3**(1): p. 35-43.