AWARD NUMBER: W81XWH-19-1-0154

TITLE: Immunological Approaches for ARID1A-Mutated Ovarian Cancer

PRINCIPAL INVESTIGATOR: Rugang Zhang

CONTRACTING ORGANIZATION: The Wistar Institute, Philadelphia, PA

REPORT DATE: January 2023

TYPE OF REPORT: Final

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
January 2023	Final	30Sep2019-29Sep2022

4. TITLE AND SUBTITLE

Immunological Approaches for ARID1A-Mutated Ovarian Cancer

5a. CONTRACT NUMBER
W81XWH-19-1-0154

5b. GRANT NUMBER
OC180109

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Rugang Zhang

E-Mail: rzhang@wistar.org

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. Our preliminary data show that *ARID1A* mutation sensitizes ovarian cancer to anti-PD-L1 treatment. Our recent studies also show that *ARID1A*-mutated ovarian cancer depends on HDAC6 activity. Although most translational studies on HDAC6 inhibitors have focused on their effects on tumor cells, emerging evidence suggests that HDAC6 inhibitors have immunomodulatory effects on various immune cellular subsets. Indeed, our preliminary data suggests that the HDAC6 inhibitor ACY1215 enhances the activation of T cells and suppresses MDSCs in *ARID1A*-mutated OCCCs. They suggest that HDAC6 inhibitors may enhance the anti-PD-L1 therapy in *ARID1A*-mutated ovarian cancers. Our **central hypothesis** is that *ARID1A*-mutated ovarian cancer can be therapeutically eradicated by a combination of clinically applicable HDAC6 inhibitor and anti-PD-L1 immune checkpoint blockade.

15. SUBJECT TERMS
Epithelial ovarian cancer, ovarian clear cell carcinoma, ARID1A, SWI/SNF, HDAC6, Immune checkpoint blockade, anti-PD-L1.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	22	19b. TELEPHONE NUMBER *(include area code)*

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. Our preliminary data show that ARID1A mutation sensitizes ovarian cancer to anti-PD-L1 treatment. Our recent studies also show that ARID1A-mutated ovarian cancer depends on HDAC6 activity. Although most translational studies on HDAC6 inhibitors have focused on their effects on tumor cells, emerging evidence suggests that HDAC6 inhibitors have immunomodulatory effects on various immune cellular subsets. Indeed, our preliminary data suggests that the HDAC6 inhibitor ACY1215 enhances the activation of T cells and suppresses MDSCs in ARID1A-mutated OCCCs. They suggest that HDAC6 inhibitors may enhance the anti-PD-L1 therapy in ARID1A-mutated ovarian cancers. Our **central hypothesis** is that ARID1A-mutated ovarian cancer can be therapeutically eradicated by a combination of clinically applicable HDAC6 inhibitor and anti-PD-L1 immune checkpoint blockade.

2. KEYWORDS:

Epithelial ovarian cancer, ovarian clear cell carcinoma, ARID1A, SWI/SNF, HDAC6, Immune checkpoint blockade, anti-PD-L1.

3. 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 immune checkpoint anti-PD-L1 and a clinically applicable HDAC6 inhibitor.

Specific Aim 1: To investigate the effects of HDAC6 inhibition and ARID1A status on tumor immune microenvironment.

Specific Aim 2: To develop a novel therapeutic approach for ARID1A-mutated ovarian cancer by combining clinically applicable HDAC6 inhibitor and anti-PD-L1 antibody.

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 two years are as following:

1. We investigated the mechanism by which ARID1A regulates anti-PD-L1 response.
2. We determined the effects of the HDAC6 inhibitor on immune modulating cells in the tumor microenvironment.
3. We determined whether HDAC6 inhibitor and anti-PD-L1 are synergistic in suppressing the growth of ARID1A-inactivated clear cell ovarian carcinoma.
4. We determined the changes in the immune modulating cells induced by the combination of the HDAC6 inhibitor and anti-PD-L1.
5. We determined whether immune modulating CD8 T cells contribute to the observed tumor suppressive effects in combination treatment.

2) specific objectives;

The major objectives in the first two years of funding are as following:

1. To elucidate how ARID1A may regulate expression of CD274 that encodes PD-L1.
2. To prolife how HDAC6 inhibition modulates immune microenvironment in ARID1A inactivated ovarian clear cell carcinomas.
3. To explore the synergy between HDAC6 inhibitor and immune checkpoint blockade in ARID1A inactivated ovarian clear cell carcinomas.
4. To characterize the changes in the immune modulating cells induced by HDAC6 inhibitor and immune checkpoint blockade in ARID1A inactivated ovarian clear cell carcinomas.
5. To study the role of CD8 T cells in mediating the observed tumor suppressive effects induced by HDAC6 inhibitor and immune checkpoint blockade combination in ARID1A inactivated ovarian clear cell carcinomas.

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

CD274 is a direct ARID1A target gene.

ARID1A chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) analysis revealed that ARID1A was associated with the PD-L1 encoding CD274 gene promoter in ARID1A wildtype OCCC cells [1] (**Figure 1A**). We validated the binding of ARID1A to the Cd274 gene promoter by ChIP in the mouse ovarian ID8-Defb29/Vegf cells (**Figure 1B-C**) in which PD-L1 is implicated [2]. As a negative control, ARID1A binding to the Cd274 promoter was reduced to a level observed in IgG controls in ARID1A knockout ID8-Defb29/Vegf cells (**Figure 1C**). Notably, SNF5, a core subunit of the SWI/SNF complex, was also associated with the Cd274 promoter and its association was reduced by ARID1A knockout (**Figure 1C**). Expression of ARID1B, the mutually exclusive subunit of the SWI/SNF complex with ARID1A, was upregulated in ARID1A knockout ID8-Defb29/Vegf cells (**Figure 1B**) [3]. Although ARID1B was also associated with the Cd274 promoter, ARID1A knockout did not affect the association of ARID1B with the Cd274 promoter (**Figure 1C**). This suggests that ARID1B is unable to compensate for ARID1A loss on the Cd274 promoter. Similar observations were also made in the ARID1A wildtype human OCCC cell lines OVCA429 and RMG1 cells (**Figure 2**), indicating that the association of ARID1A with the CD274 promoter is not a cell line-specific effect. Together, we conclude that CD274 is a direct ARID1A target gene.

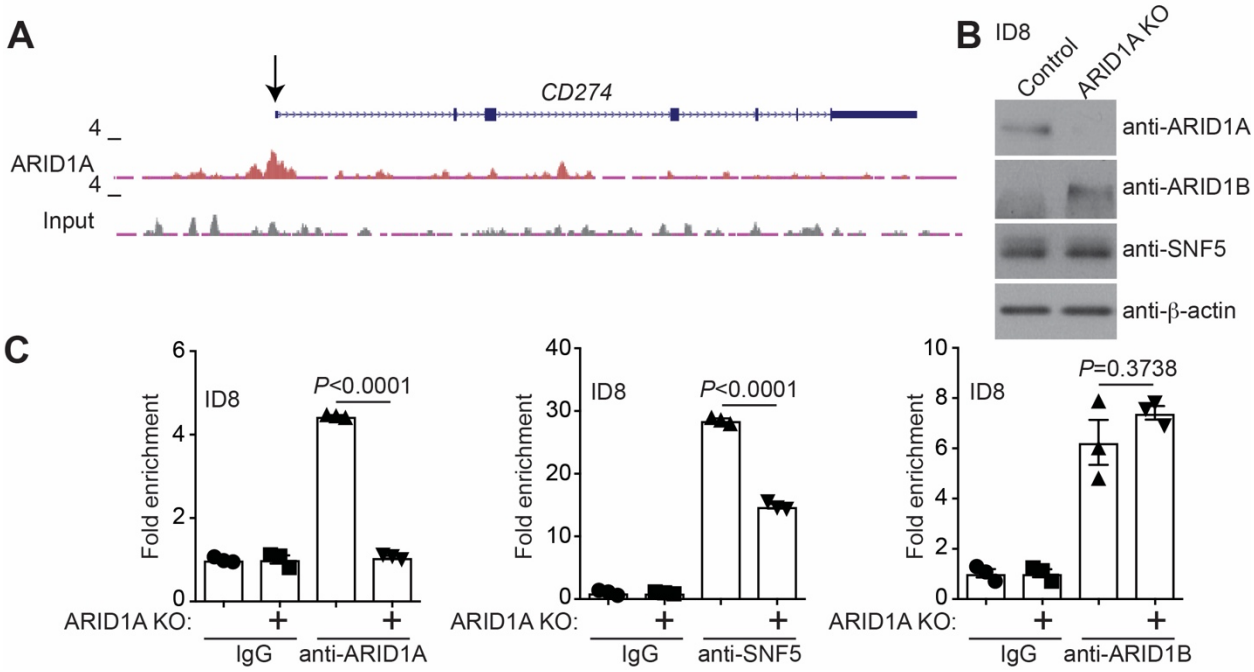

Figure 1. CD274 is a direct ARID1A target gene in mouse ovarian cancer cells.
(**A**) ARID1A ChIP-seq track on the CD274 gene locus in ARID1A wildtype RMG1 cells. (**B**) Expression of ARID1A, ARID1B, SNF5 and □-actin in the ARID1A wildtype control and ARID1A knockout mouse ovarian ID8-Defb29/Vegf cells. (**C**) The indicated ID8-Defb29/Vegf cells were subjected to ChIP analysis for the association of the indicated proteins with the Cd274 gene promoter using the indicated antibodies against ARID1A, SNF5, ARID1B or an isotype-matched IgG control. Error bars represent ± S.E.M. n= 3 independent experiments.

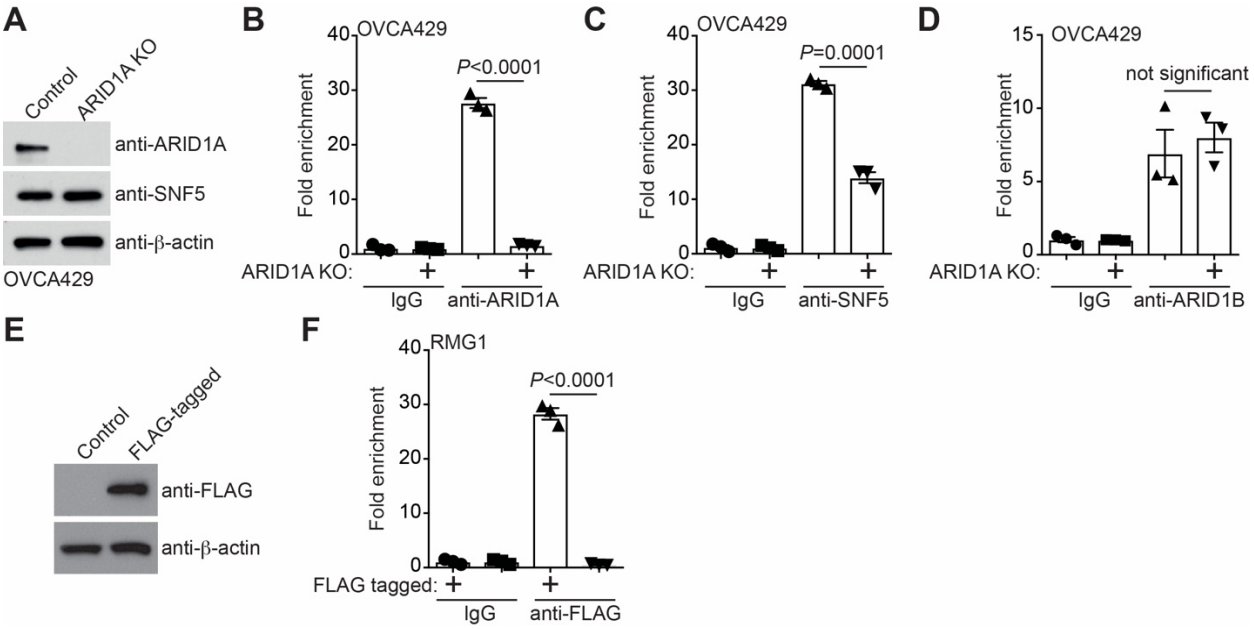

Figure 2. CD274 is a direct ARID1A target gene in human ovarian clear cell ovarian cancer cells.
(**A**) Expression of ARID1A and SNF5 in the ARID1A wildtype control and ARID1A knockout human OVCA429 OCCC cells. β-actin expression was used as a loading control. (**B-D**) ARID1A wildtype

control and ARID1A knockout OVCA429 OCCC cells were subjected to analysis for the association of the indicated proteins with the CD274 gene promoter using the indicated antibodies against ARID1A (B) (by CUT & RUN), SNF5 (C) or ARID1B (D) (by ChIP) (C). An isotype-matched IgG was used as a negative control. (**E-F**) Endogenously FLAG-tagged ARID1A wildtype RMG1 OCCC cells were subjected to immunoblot analysis for expression of FLAG (E) or for the association of FLAG-ARID1A with the CD274 gene promoter by ChIP analysis using an anti-FLAG antibody (F). An isotype-matched IgG was used as a negative control. Error bars represent ± S.E.M. n= 3 independent experiments.

ARID1A represses CD274 gene transcription.

We next determined the effect of ARID1A status on changes in Cd274 mRNA and PD-L1 expression. Compared with ARID1A wildtype control ID8-Defb29/Vegf cells, Cd274 mRNA was increased by ARID1A knockout (**Figure 3A**). Consistently, PD-L1 expression measured by both immunoblot and fluorescence-activated cell sorting (FACS) analysis was upregulated upon ARID1A knockout (**Figure 3A**). Interferon-gamma (IFNg) plays a major role in inducing PD-L1 expression [4]. Thus, we examined the effects of ARID1A knockout on IFNg-induced PD-L1 expression. ARID1A knockout significantly enhanced the upregulation of Cd274 mRNA and PD-L1 expression induced by IFNg treatment (**Figure 3A**). Similar findings were made in both ARID1A wildtype mouse ID8-Defb29/Vegf cells and human OVCA429 and RMG1 cells with or without ARID1A knockout (**Figure 4**). We next examined the association of RNA polymerase II (Pol II) and lysine 4 trimethylated histone H3 (H3K4me3), a transcription active promoter epigenetic mark, with the Cd274 promoter. Consistent with changes observed in Cd274 mRNA and PD-L1 expression, ARID1A knockout enhanced the association of Pol II and H3K4me3 with the Cd274 promoter with or without IFNg stimulation (**Figure 3B**). Together, we conclude that ARID1A represses CD274 gene transcription at both the basal levels and in response to IFNg stimulation.

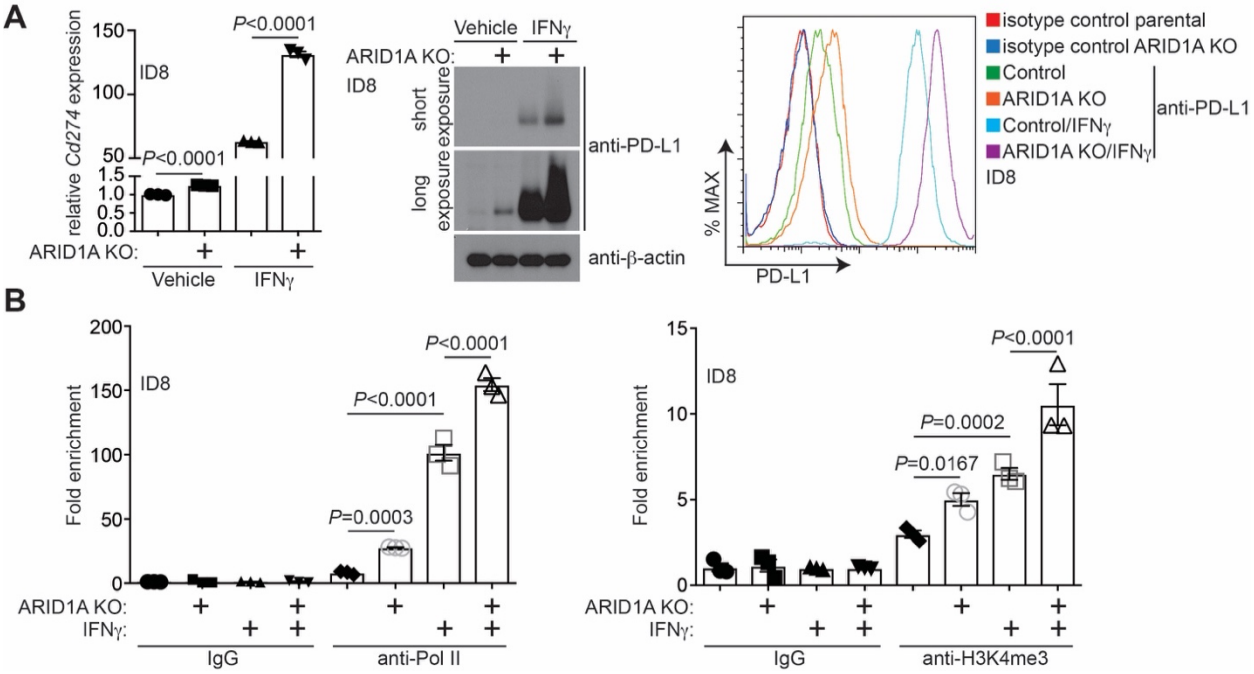

Figure 3. ARID1A transcriptionally represses Cd274 in mouse ovarian cancer cells. (**A**) Expression of Cd274 mRNA and PD-L1 protein in ARID1A wildtype control and ARID1A knockout mouse ovarian ID8-Defb29/Vegf cells treated with or without 20 ng/ml IFNγ determined by qRT-PCR (left) or immunoblot (middle). The cell surface expression of PD-L1 in these cells was

determined by flow cytometry analysis (right). (**B**) The indicated ID8-Defb29/Vegf cells treated with or without 20 ng/ml IFNγ cells were subjected to ChIP analysis for the Cd274 gene promoter using the indicated antibodies or an isotype-matched IgG control. Error bars represent ± S.E.M. n= 3 independent experiments.

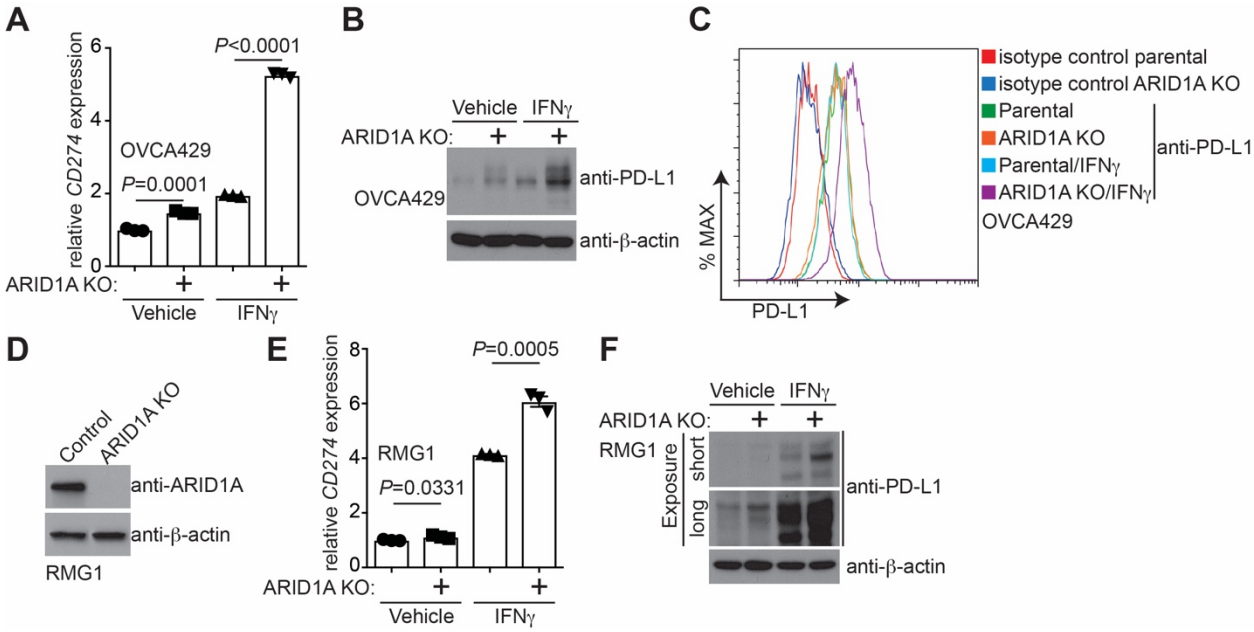

Figure 4. ARID1A represses CD274 transcription in human ovarian clear cell cancer cells. (**A-B**) Expression of CD274 mRNA (A) and PD-L1 protein (B) in ARID1A wildtype control and ARID1A knockout OVCA429 OCCC cells treated with or without 20 ng/ml IFN determined by qRT-PCR or immunoblot, respectively. (**C**) The cell surface expression of PD-L1 in these cells was determined by flow cytometry analysis. (**E-G**) Validation of ARID1A knockout by immunoblot in RMG1 OCCC cells (E). Expression of CD274 mRNA (F) and PD-L1 protein (G) in ARID1A wildtype control and ARID1A knockout RMG1 OCCC cells treated with or without 20 ng/ml IFN determined by qRT-PCR or immunoblot, respectively. Error bars represent ± S.E.M. n= 3 independent experiments.

HDAC6 inhibitor boosts antitumor immunity.

Given HDAC6 inhibitors' role in immune modulation [5-7], we examined the effects of HDAC6 inhibitor ACY1215 in a conditional genetic ARID1A$^{flox/flox}$/PIK3CA^{H1047R} OCCC mouse model [8, 9] (**Figure 5A**). Notably, HDAC6 inhibitor ACY1215 significantly increased the CD69^{+} activated CD4 and CD8 T cells in the peritoneal wash (**Figure 5B**). Consistently, IFNg^{+} CD4 and CD8 T cells were also significantly increased by ACY1215 treatment (**Figure 5A**). In contrast, ACY1215 did not significantly affect Granzyme B^{+} CD8 T cells or Foxp3^{+} regulatory T cells (**Figure 5C-D**). These findings suggest that HDAC6 inhibition may boost antitumor immunity.

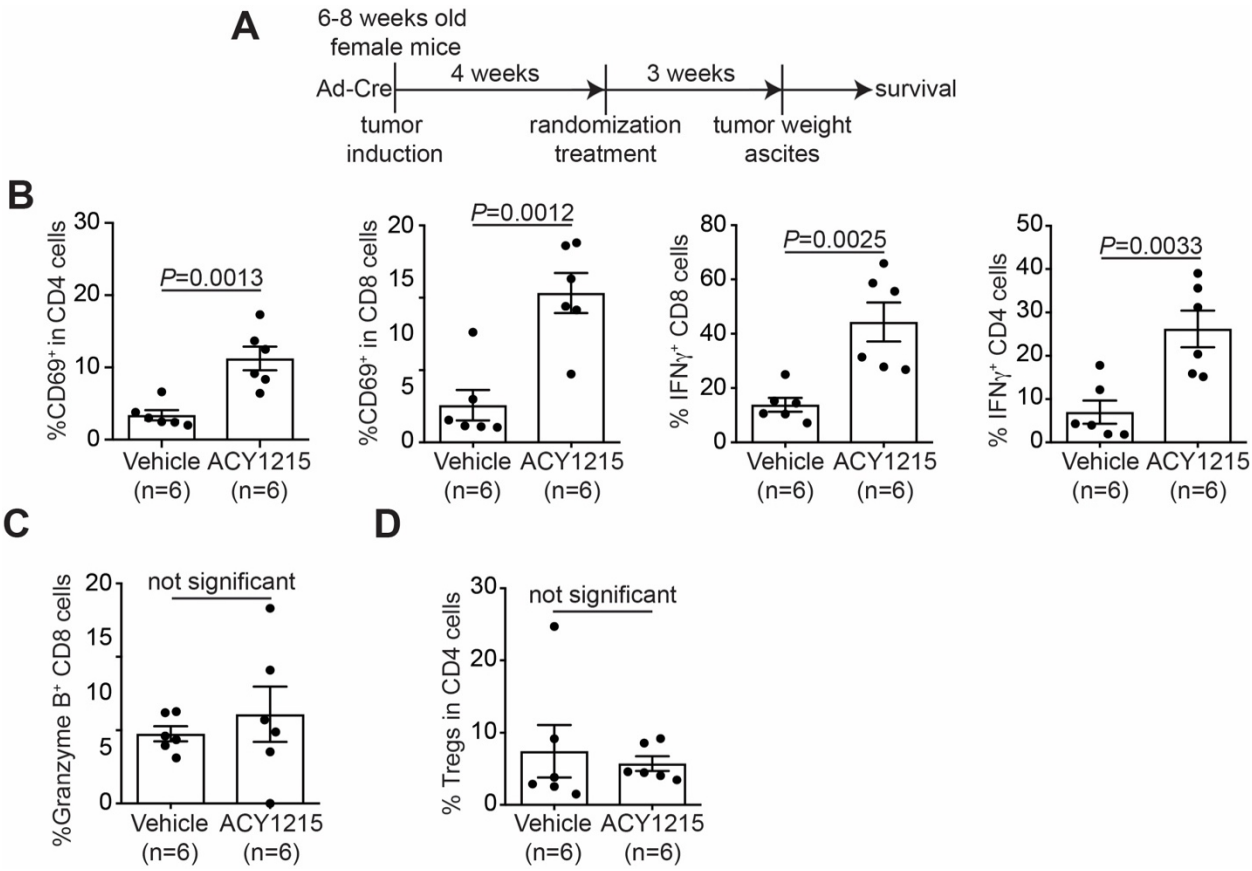

Figure 5. ACY1215 boosts antitumor immunity.
ARID1A$^{flox/flox}$/PIK3CA^{H1047R} OCCCs were induced by intrabursal adenovirus-encoded Cre injection and allowed to establish for four weeks (**A**). The mice were randomized to treat with vehicle control or ACY1215 (50 mg/kg daily by i.p.) for an additional three weeks. At the end of treatment, percentage of CD69 or IFN□ positive CD8 and CD4 T cells (**B**), Granzyme B$^+$ CD8 T cells (**C**), or Foxp3$^+$ regulatory CD4 T cells (**D**) was assessed by flow cytometry in the peritoneal wash.

Combination of HDAC6 inhibitor and anti-PD-L1 in the ARID1A$^{flox/flox}$/PIK3CA^{H1047R} OCCC mouse model.

Since ARID1A directly represses PD-L1 and HDAC6 inhibition increases T cell activation and activity, we sought to determine the effects of HDAC6 inhibitor ACY1215 and anti-PD-L1 combination in ARID1A-inactivated OCCCs. Toward this goal, we first established OCCCs in 6-8 week old ARID1A$^{flox/flox}$/PIK3CA^{H1047R} female mice by intrabursally injecting adenovirus-Cre [8]. Four weeks after the adenovirus-Cre injection, the mice were randomized into four treatment groups: 1) vehicle and IgG control; 2) ACY1215 (50 mg/kg daily by i.p.) and IgG control; 3) vehicle control and anti-PD-L1 antibody (10 mg/kg twice weekly by i.p.); and 4) ACY1215 and anti-PD-L1 antibody combination for an additional three weeks. At the end of treatment, orthotopic tumors were surgically removed (**Figure 6A**). The tumor weight was measured as a surrogate for tumor burden. As previously reported [8, 10], both anti-PD-L1 antibody and ACY1215 significantly reduced the tumor weight in the OCCC model (**Figure 6A**). We also examined effects of the ACY1215 and anti-PD-L1 combination in reducing ascites produced in the Arid1a$^{flox/flox}$/Pik3ca^{H1047R} OCCC model. Both ACY1215 and anti-PD-L1 single treatment significantly reduced the amount of ascites produced in this model (**Figure 6B**). The reduction in tumor weight and ascites production by ACY1215 or anti-PD-L1 single treatment correlated with an improvement of survival (**Figure 6C**). The HDAC6 inhibitor ACY1215 and anti-PD-L1 combination was synergistic in reducing the

tumor burden and improving the survival of tumor-bearing mice (**Figure 6A and C**). Notably, the combination completely eliminated the ascites production (**Figure 6D**). The doses of ACY1215 and anti-PD-L1 used in this study did not significantly affect the body weight of treated mice (**Figure 6E**), suggesting that effective combination doses can be achieved without gross toxicity. Together, we conclude that HDAC6 inhibitor ACY1215 and anti-PD-L1 are synergistic in reducing tumor burden, which correlates with an improvement of survival of mice bearing ARID1A-inactivated OCCCs.

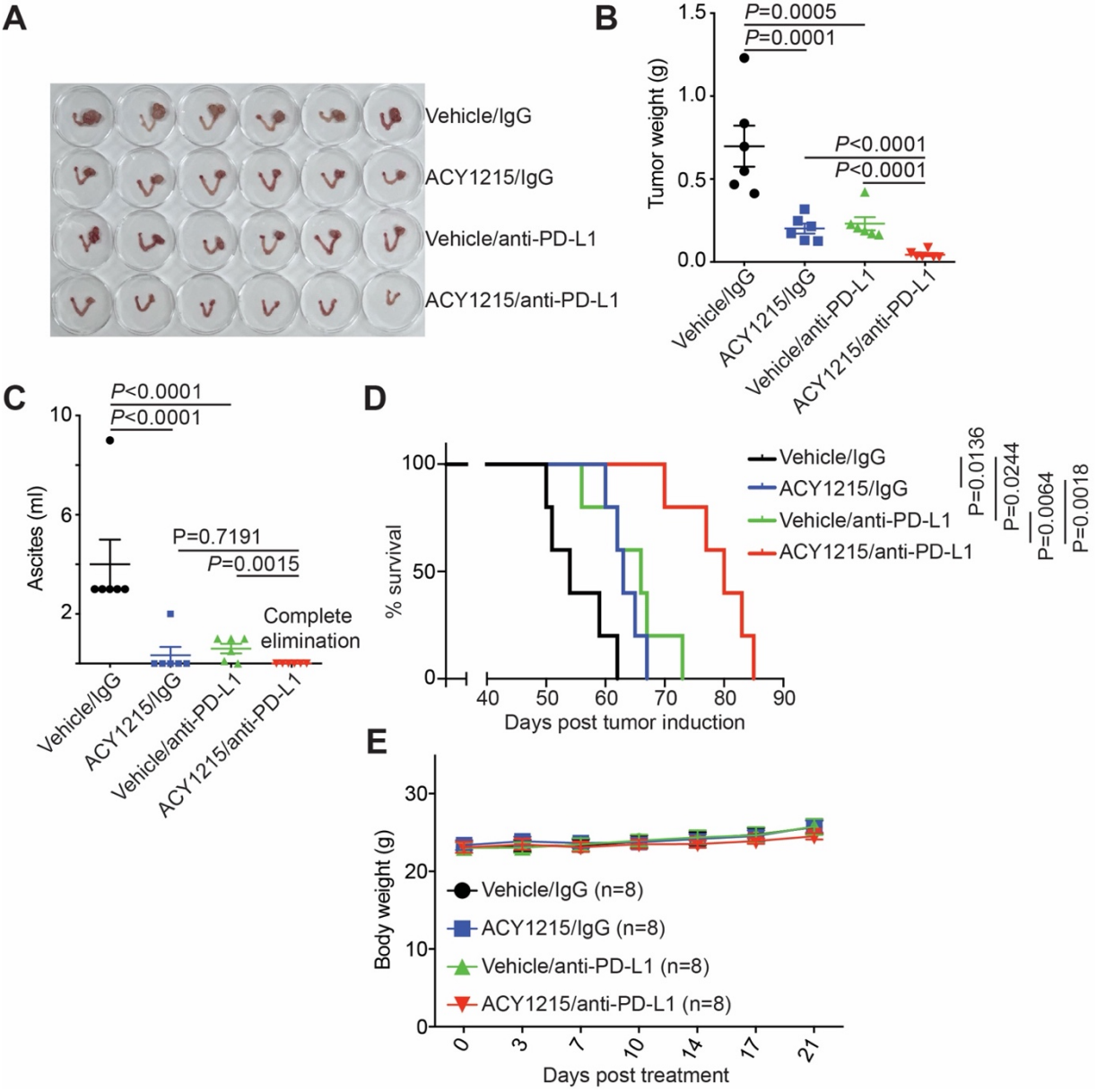

Figure 6. ACY1215 and anti-PD-L1 are synergistic in limiting tumor progression in vivo. (**A**) ARID1A$^{flox/flox}$/PIK3CA^{H1047R} OCCCs were induced by intrabursal adenovirus-encoded Cre injection and allowed to establish for four weeks. The mice were randomized into 4 indicated treatment groups and treated for an additional three weeks. At the end of treatment, 6 mice from each of the indicated groups were euthanized. Images of dissected reproductive tracks with tumors were shown. (**B**) The weights of the dissected tumors were quantified as a surrogate for tumor burden. (**C**) Same as (B), but quantified for the ascites produced. (**D**) After completing

treatment, the mice were followed for survival and shown are the Kaplan–Meier survival curves for each of the indicated groups. (**E**) The body weight of mice in the indicated treatment groups during 3 weeks of treatment. Error bars represent ± S.E.M.

We next sought to determine the effects of combination on immune infiltration. Notably, HDAC6 inhibitor ACY1215 significantly increased the $CD69^+$ activated CD4 and CD8 T cells in the peritoneal wash (**Figure 7A**). Consistently, IFN γ^+ CD4 and CD8 T cells were also significantly increased by ACY1215 treatment (**Figure 7B**). In contrast, ACY1215 did not significantly affect Granzyme B^+ CD8 T cells or $Foxp3^+$ regulatory T cells (**Figure 7C**). However, a combination of ACY1215 and anti-PD-L1 treatment only increased $IFN\gamma^+$ CD8, but not CD4 T cells (**Figure 7B**). This suggests the implication of CD8 T cells in the combination treatment.

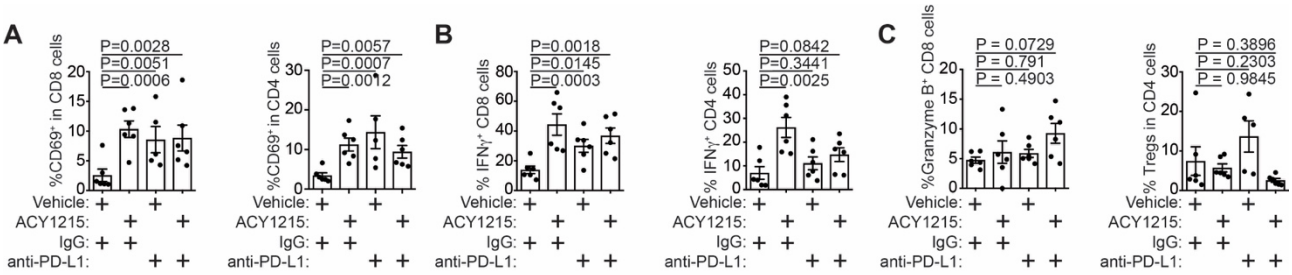

Figure 7. ACY1215 and anti-PD-L1 treatment increased IFNγ+ CD8 T cells.
(**A**) $ARID1A^{flox/flox}$/$PIK3CA^{H1047R}$ OCCCs were induced by intrabursal adenovirus-encoded Cre injection and allowed to establish for four weeks. The mice were randomized into 4 indicated treatment groups and treated for an additional three weeks. At the end of treatment, percentage of CD69+ CD8 and CD4 T cells (**A**), IFN γ positive CD8 and CD4 T cells (**B**), and Granzyme B positive CD8 cells and intracellular FOXP3 positively stained regulatory CD4 T cells (Treg) (**C**) were assessed by flow cytometry in the peritoneal wash. Error bars represent ± S.E.M.

$CD8^+$ T cell depletion abrogates the antitumor effects of ACY1215 and anti-PD-L1 combination.

Since ACY1215 and anti-PD-L1 combination increases $IFN\gamma^+$ CD8, but not CD4, T cells (**Figure 7**) and cytotoxic CD8 T cells play a critical role in mediating the antitumor effects of anti-PD-L1 treatment [11], we next sought to determine whether the combination limits the progression of *ARID1A*-mutated OCCCs through CD8 T cells. Towards this goal, we depleted CD8 T cells by treating the combination-treated mice with an anti-CD8 antibody (**Figure 8A**). Compared with IgG control-treated mice, anti-CD8 antibody significantly abrogated the observed reduction in tumor weight and ascites production induced by the combination (**Figure 8B-D**). Consistently, the improvement of survival observed in the combination treatment group was also abrogated by the anti-CD8 antibody (**Figure 8E**). However, anti-CD8 antibody did not significantly reduce the CD4 T cell activation (**Figure 8F**). This result indicates that T cell activation induced by ACY1215 is not merely a reflection of reduction in tumor burden in the treated mice. Together, these results support that the observed antitumor effects in ARID1A-inactivated OCCCs by ACY1215 and anti-PD-L1 combination is CD8 cytotoxic T cell dependent.

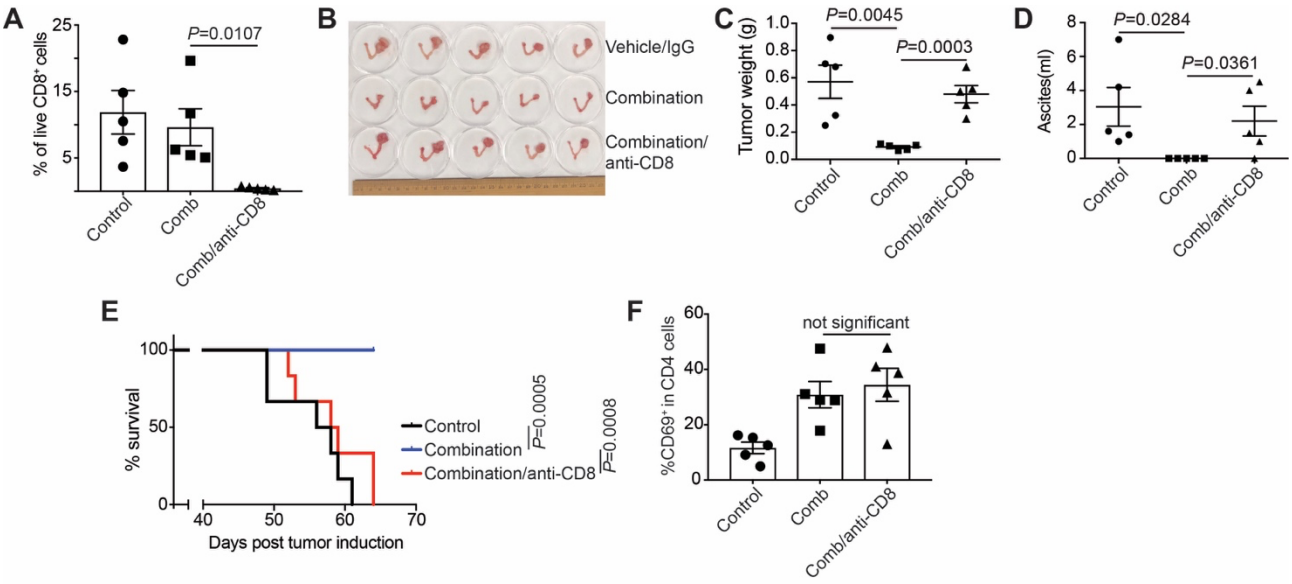

Figure 8. Depletion of CD8$^+$ T cells abrogates the antitumor effects of ACY1215 and anti-PD-L1 combination.
(**A**) *ARID1A$^{flox/flox}$/PIK3CA^{H1047R}* OCCCs were induced by intrabursal adenovirus-encoded Cre injection and allowed to establish for four weeks. The mice were randomized into three indicated experimental groups (n=5 mice/group). The depletion of CD8$^+$ T cells was confirmed by flow cytometry analysis of blood cells collected via the retro-orbital vein. (**B-D**) Images of dissected reproductive tracks with tumors were shown (**B**), the weights of the dissected tumors were quantified as a surrogate for tumor burden (**C**), and ascites produced were quantified (**D**). (**E**) After three weeks treatment, the mice were followed for survival and shown are the Kaplan–Meier survival curves. (**F**) At the end of treatment, percentage of CD69 positive CD4 T cells was assessed by flow cytometry in the dissected tumors. Error bars represent ± S.E.M.

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?
Nothingto report. The stated goals have all been achieved during the funding period. This award is terminated with the present final closeout report.

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:

Nothing to report.

Changes in approach and reasons for change
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report.

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

Journal publications.

1. Zhou W, Liu H, Yuan Z, Zundell J, Towers M, Lin J, Lombardi S, Nie H, Kossenkov AV, Drapkin R, Montaner LJ, Wu S, **Zhang R**. Targeting the mevalonate pathway suppresses ARID1A inactivated cancers by driving pyroptosis. ***Cancer Cell***, under review.
2. Hao X, Zhao B, Towers M, Liao L, Tang HS, Havas A, Kossenkov AV, **Berger S**, **Adams PD**, Speicher DW, **Zhang R**. TXNRD1 drives innate immune response in senscent cells and promotes age-associated inflammation. ***Nature Cell Biology***, under review.
3. Liu H, Lin J, Zhou W, Moses R, Dai Z, Kossenkov A, Drapkin R, Bitler BG, Karakshev S, **Zhang R** (2022). KDM5A inhibts anti-tumor immune response thorugh downregulation of antigen presentation pathway in ovarian cancer. ***Cancer Immunology Research***, 10(8): 1028-1038. PMCID: PMC9357105

4. Lin J, Guo D, Liu H, Zhou W, Wang C, Muller I, Kossenkov AK, Drapkin R, Bitler BG, Helin K, **Zhang R** (2021). SETDB1-TRIM28 complex suppresses antitumor immunity. ***Cancer Immunology Research***, 9(12): 1413-1424. PMCID: PMC8647838.

5. Zundell J, Fukumoto T, Lin J, Fatkhudinov N, Nacarelli T, Kossenkov AV, Liu Q, Cassel J, Hu CC, Wu S, **Zhang R** (2021). Targeting the IRE1a/XBP1 endoplasmic reticulum stress response pathway in ARID1A-mutant ovarian cancers. ***Cancer Research***, 81(20): 5325-5335. PMCID: PMC8723353.

6. Lin J, Fukumoto T, Zundell J, Liu H, Yan Q, Tang CHA, Wu S, Zhou W, Karakashev S, Hu CCA, Sarma K, Kossenkov AV, **Zhang R** (2021). CARM1 determines endoplasmic reticulum stress response by controlling XBP1. **Nature Communications**, 12 (1): 5321. PMCID: PMC8423755.

7. Liu P, Li F, Lin J, Fukumoto T, Nacarelli T, Hao X, Kossenkov AV, Simon MC, **Zhang R** (2021). m^6A independent genome-wide METTL3 and METTL14 redistribution drives senescence-associated secretory phenotype**. Nature Cell Biology**, 23(4): 355-365. PMCID: PMC8035315.

8. Wu S, Fukumoto T, Lin J, Nacarelli T, Wang Y, Ong D, Liu H, Fatkhutdinov N, Zundell JA, Karakashev S, Zhou W, Schwartz LE, Tang HY, Drapkin R, Liu Q, Huntsman DG, Kossenkov AV, Speicher DW, Schug ZT, Dang CV, **Zhang R** (2021). Therapeuitc targeting glutamine dependence in SWI/SNF-inactivated cancers. **Nature Cancer**, 2: 189-200. PMCID: PMC8168620.

9. Kim H, Xu H, George E, Hallberg D, Kumar S, Jagannathan V, Medvedev S, Kinose Y, Devins K, Verma P, Ly K, Wang Y, Greenberg RA, Schwartz L, Johnson N, Scharpf RB, Mills GB, Zhang R, Velculescu VE, Brown EJ, Simpkins F (2020) Combining PARP and ATR inhibition overcomens PARP inhibitor and platinum resistance in ovarian cancer models. **Nature Communications**, 11: 3726. PMCID: PMC7381609.

10. Hashimoto A, Fukumoto T, **Zhang R**, Gabrilovich D (2020) Selective targeting of different populations of myeloid-derived suppressor cells by histone deacetylase inhibitors. **Cancer Immunology and Immunotherapy**, in press.

11. Karakashev S, Fukumoto T, Zhao B, Lin J, Wu S, Fatkhutdinov N, Park PW, Semenova G, Jean S, Gadungog MG, Borowsky ME, Kossenkov AV, Liu Q, **Zhang R** (2020) EZH2 inhibition sensitizes CARM1-high, homologous recombination proficient ovarian cancers to PARP inhibition. **Cancer Cell** 37: 157-167. PMCID: PMC7155421.

12. Zhao B, Liu P, Fukumoto T, Nacarelli T, Fatkhutdinov N, Wu S, Lin J, Aird KM, Tang SY, Liu Q, Speicher DW, **Zhang R** (2020) Topoisomerase 1 cleavage complex enables pattern recongnition and inflammation during senescence. **Nature Communications**, 11: 908. PMCID: PMC7031389.

13. Nacarelli T, Fukumoto T, Zundell JA, Fatkhutdinov N, Jean S, Cadungog MG, Borowsky ME, **Zhang R** (2020) NAMPT inhibition suppresses cancer stem-like cells associated with therapy-induced senescence in ovarian cancer. **Cancer Research** 80: 890-900. PMCID: PMC7024650.

14. Wu S, Fatkhutdinov N, Rosin L, Luppino JM, Iwasaki O, Tanizawa H, Tang HY, Kossenkov AV, Gardini A, Noma KI, Speicher DW, Joyce EF, **Zhang R** (2019) ARID1A spatially partitions interphase chromosomes. **Science Advances** 5: eaaw5294. PMCID: PMC6531001.

15. Fukumoto T, Fatkhutdinov N, Zundell JA, Tcyganov EN, Nacarelli T, Karakashev S, Wu S, Liu Q, Gabrilovich DI, **Zhang R** (2019) HDAC6 inhibition synergizes with anti-PD-L1 therapy in ARID1A-inactivated ovarian cancer. **Cancer Research** 21: 5482-5489. PMCID: PMC6825538.

16. Zhao B, Lin J, Rong L, Wu S, Deng Z, Fatkhutdinov N, Zundell J, Fukumoto T, Liu Q, Kossenkov A, Jean S, Cadungog MG, Borowsky ME, Drapkin R, Lieberman PM, Abate-Shen CT, **Zhang R** (2019) ARID1A promotes genomic stability through protecting telomere cohesion. **Nature Communications** 10: 4067. PMCID: PMC6731242.

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
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Rugang Zhang
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-7255-2360
Nearest person month worked:	.6 cm
Contribution to Project:	Supervised the study.
Funding Support:	This award

Name:	Dajiang Guo
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.7 cm
Contribution to Project:	Performed the study.
Funding Support:	This award

Name:	Heng Liu
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	10.2 cm
Contribution to Project:	Performed the study.
Funding Support:	This award

Name:	Boyi Zhang
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3.2 cm
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?

The following changes have occurred in other support since the last reporting period:

Dr. Rugang Zhang:

Deactivation of "Developing Epigenetic Therapies for Ovarian Cancer" as of 12/2021
Activation of "Metabolic basis of ARID1A-mutated ovarian cancer" as of 08/2022
Activation of "Therapeutic Targeting Mevalonate Pathway in ARID1A-Mutated Ovarian Cancer" as of 09/2022.
Dr. Qin Liu:

Termination of "BEAT-HIV: Delaney Collaboratory to Cure HIV1 Infection by Combination Immunotherapy"
Termination of "Targeting TERT in Melanoma"
End Participation in Chromatin basis of cellular senescence and its implication in epithelial ovarian cancer"
Termination of "Role of Intestinal Barrier Integrity and Antibody Glycosylation in Long-COVID During HIV-infection"
Activation of "Mechanistic basis and therapeutic strategies for ARID1A mutation in ovarian cancer"
Activation of "A First-in-Human Phase 1 Clinical Trial of Mitochondrial-Targeted Hsp90 Inhibitor, Gamitrinib"
Activation of "Host Glycomic Modulation of HIV-associated Neuro-inflammation During Viral Suppression"
Activation of "Gamma delta T cell based melanoma therapies"
Activation of Targeting S6k2 to Overcome Drug Resistance in NRAS-Mutant Melanoma"
Activation of "Host Glycomic and Metabolic Modulators of Inflammation During HIV Treatment Interruptions"
Activation of "Metabolic basis of ARID1A-mutated ovarian cancer"
Activation of "Therapeutic Targeting Mevalonate Pathway in ARID1A-Mutated Ovarian Cancer"
Activation of "New control of oncogene activation in T-cell leukemia"
Activation of Microbiota-Mediated Bidirectional Interactions Between Alcohol Misuse and Post-COVID-19 Syndrome"

What other organizations were involved as partners?
Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

- Award Chart
- Award Expiration Transition Plan

OC180109: Immunological approaches for ARID1A-mutated ovarian cancer

PI: Rugang Zhang, Wistar, PA
Budget: $803,700.00
Topic Area: OCRP- Investigator-Initiated Research Award
Mechanism: W81XWH-18-OCRP-IIRA

Research Area(s): 1st 0105 - Tumor Suppressor Genes, 2nd 0805 - Targeted Therapies
Award Status: 09/30/2019-09/29/2022

Study Goals:

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. Our preliminary data show that *ARID1A* mutation sensitizes ovarian cancer to anti-PD-L1 treatment. Our recent studies also show that *ARID1A*-mutated ovarian cancer depends on HDAC6 activity. Although most translational studies on HDAC6 inhibitors have focused on their effects on tumor cells, emerging evidence suggests that HDAC6 inhibitors have immunomodulatory effects on various immune cellular subsets. Indeed, our preliminary data suggests that the HDAC6 inhibitor ACY1215 enhances the activation of T cells and suppresses MDSCs in *ARID1A*-mutated OCCCs. They suggest that HDAC6 inhibitors may enhance the anti-PD-L1 therapy in *ARID1A*-mutated ovarian cancers. Our **central hypothesis** is that *ARID1A*-mutated ovarian cancer can be therapeutically eradicated by a combination of clinically applicable HDAC6 inhibitor and anti-PD-L1 immune checkpoint blockade.

Specific Aims:

Specific Aim 1: To investigate the effects of HDAC6 inhibition and ARID1A status on tumor immune microenvironment.
Specific Aim 2: To develop a novel therapeutic approach for ARID1A-mutated ovarian cancer by combining clinically applicable HDAC6 inhibitor and anti-PD-L1 antibody.

OC180109: Immunological approaches for ARID1A-mutated ovarian cancer

PI: Rugang Zhang, Wistar, PA **Budget:** $803,700.00

Topic Area: OCRP- Investigator-Initiated Research Award **Mechanism:** W81XWH-18-OCRP-IIRA

Key Accomplishments and Outcomes:

1. **Publications:** Zhou W, Liu H, Yuan Z, Zundell J, Towers M, Lin J, Lombardi S, Nie H, Kossenkov AV, Drapkin R, Montaner LJ, Wu S, **Zhang R**. Targeting the mevalonate pathway suppresses ARID1A inactivated cancers by driving pyroptosis. ***Cancer Cell***, under review.
2. Hao X, Zhao B, Towers M, Liao L, Tang HS, Havas A, Kossenkov AV, **Berger S**, **Adams PD**, Speicher DW, **Zhang R**. TXNRD1 drives innate immune response in senscent cells and promotes age-associated inflammation. ***Nature Cell Biology***, under review.
3. Liu H, Lin J, Zhou W, Moses R, Dai Z, Kossenkov A, Drapkin R, Bitler BG, Karakshev S, **Zhang R** (2022). KDM5A inhibts anti-tumor immune response thorugh downregulation of antigen presentation pathway in ovarian cancer. ***Cancer Immunology Research***, 10(8): 1028-1038. PMCID: PMC9357105
4. Lin J, Guo D, Liu H, Zhou W, Wang C, Muller I, Kossenkov AK, Drapkin R, Bitler BG, Helin K, **Zhang R** (2021). SETDB1-TRIM28 complex suppresses antitumor immunity. ***Cancer Immunology Research***, 9(12): 1413-1424. PMCID: PMC8647838.
5. Zundell J, Fukumoto T, Lin J, Fatkhudinov N, Nacarelli T, Kossenkov AV, Liu Q, Cassel J, Hu CC, Wu S, **Zhang R** (2021). Targeting the IRE1a/XBP1 endoplasmic reticulum stress response pathway in ARID1A-mutant ovarian cancers. ***Cancer Research***, 81(20): 5325-5335. PMCID: PMC8723353.
6. Lin J, Fukumoto T, Zundell J, Liu H, Yan Q, Tang CHA, Wu S, Zhou W, Karakashev S, Hu CCA, Sarma K, Kossenkov AV, **Zhang R** (2021). CARM1 determines endoplasmic reticulum stress response by controlling XBP1. **Nature Communications**, 12 (1): 5321. PMCID: PMC8423755.
7. Liu P, Li F, Lin J, Fukumoto T, Nacarelli T, Hao X, Kossenkov AV, Simon MC, **Zhang R** (2021). m^6A independent genome-wide METTL3 and METTL14 redistribution drives senescence-associated secretory phenotype**.** **Nature Cell Biology**, 23(4): 355-365. PMCID: PMC8035315.
8. Wu S, Fukumoto T, Lin J, Nacarelli T, Wang Y, Ong D, Liu H, Fatkhutdinov N, Zundell JA, Karakashev S, Zhou W, Schwartz LE, Tang HY, Drapkin R, Liu Q, Huntsman DG, Kossenkov AV, Speicher DW, Schug ZT, Dang CV, **Zhang R** (2021). Therapeuitc targeting glutamine dependence in SWI/SNF-inactivated cancers. **Nature Cancer**, 2: 189-200. PMCID: PMC8168620.
9. Kim H, Xu H, George E, Hallberg D, Kumar S, Jagannathan V, Medvedev S, Kinose Y, Devins K, Verma P, Ly K, Wang Y, Greenberg RA, Schwartz L, Johnson N, Scharpf RB, Mills GB, Zhang R, Velculescu VE, Brown EJ, Simpkins F (2020) Combining PARP and ATR inhibition overcomens PARP inhibitor and platinum resistance in ovarian cancer models. **Nature Communications**, 11: 3726. PMCID: PMC7381609.

10. Hashimoto A, Fukumoto T, **Zhang R**, Gabrilovich D (2020) Selective targeting of different populations of myeloid-derived suppressor cells by histone deacetylase inhibitors. **Cancer Immunology and Immunotherapy**, in press.
11. Karakashev S, Fukumoto T, Zhao B, Lin J, Wu S, Fatkhutdinov N, Park PW, Semenova G, Jean S, Gadungog MG, Borowsky ME, Kossenkov AV, Liu Q, **Zhang R** (2020) EZH2 inhibition sensitizes CARM1-high, homologous recombination proficient ovarian cancers to PARP inhibition. **Cancer Cell** 37: 157-167. PMCID: PMC7155421.
12. Zhao B, Liu P, Fukumoto T, Nacarelli T, Fatkhutdinov N, Wu S, Lin J, Aird KM, Tang SY, Liu Q, Speicher DW, **Zhang R** (2020) Topoisomerase 1 cleavage complex enables pattern recongnition and inflammation during senescence. **Nature Communications**, 11: 908. PMCID: PMC7031389.
13. Nacarelli T, Fukumoto T, Zundell JA, Fatkhutdinov N, Jean S, Cadungog MG, Borowsky ME, **Zhang R** (2020) NAMPT inhibition suppresses cancer stem-like cells associated with therapy-induced senescence in ovarian cancer. **Cancer Research** 80: 890-900. PMCID: PMC7024650.
14. Wu S, Fatkhutdinov N, Rosin L, Luppino JM, Iwasaki O, Tanizawa H, Tang HY, Kossenkov AV, Gardini A, Noma KI, Speicher DW, Joyce EF, **Zhang R** (2019) ARID1A spatially partitions interphase chromosomes. **Science Advances** 5: eaaw5294. PMCID: PMC6531001.
15. Fukumoto T, Fatkhutdinov N, Zundell JA, Tcyganov EN, Nacarelli T, Karakashev S, Wu S, Liu Q, Gabrilovich DI, **Zhang R** (2019) HDAC6 inhibition synergizes with anti-PD-L1 therapy in ARID1A-inactivated ovarian cancer. **Cancer Research** 21: 5482-5489. PMCID: PMC6825538.
16. Zhao B, Lin J, Rong L, Wu S, Deng Z, Fatkhutdinov N, Zundell J, Fukumoto T, Liu Q, Kossenkov A, Jean S, Cadungog MG, Borowsky ME, Drapkin R, Lieberman PM, Abate-Shen CT, **Zhang R** (2019) ARID1A promotes genomic stability through protecting telomere cohesion. **Nature Communications** 10: 4067. PMCID: PMC6731242.

Patents: none to date

Funding Obtained: none to date

Transition Plan Questionnaire

Directions: Please answer all questions that apply for each product under development. Please fill out one document per product.
This is not an application for funding; however, answers will help us understand the outcomes and products from your award.

1. After the award closes, would you be willing to periodically provide voluntary information (via email) regarding the project status (i.e. where the research is headed)? **Yes** ☑ or **No** ☐
These responses will help CDMRP demonstrate the return on its investments and will help demonstrate that the CDMRP is a responsible and successful steward of federal research funding.

2. What **conclusion(s)** does your final data support?

Our data support the following conclusions: Inhibition of HDAC6 synergizes with anti-PD-L1 immune-checkpoint blockade in ARID1A-inactivated ovarian cancer. ARID1A directly repressed transcription of CD274, the gene encoding PD-L1. Reduced tumor burden and improved survival was observed in an ARID1A inactivated OCCC mouse model treated with the HDAC6 inhibitor ACY1215 and anti-PD-L1 immune-checkpoint blockade as a result of activation and increased presence of interferon-gamma positive CD8 T cells. We confirmed that the combined treatment limited tumor progression in a cytotoxic T-cell-dependent manner as depletion of CD8+ T cells abrogated these antitumor effects. Together, these findings indicate that combined HDAC6 inhibition and immune-checkpoint blockade represents a potential treatment strategy for ARID1A-mutated cancers.

3. Will you/have you applied for/obtained follow-on-funding for this project? **If yes**, please list (a) funding organization, (b) total budget requested/obtained, and (c) title of the funded proposal. *This information will be recorded as an outcome to this award*.

Not applicable.

4. What will be **the next step(s)** for this project?

We will explore opportunities to translate the findings made by this award to clinical trials.

5. How would you classify your **lead candidate product**? a
(a) Therapeutic (Small Molecule, Biologic, Cell/Gene Therapy): Biologic
(b) Diagnostic
(c) Device
(d) Research Tool to Address a Research Bottleneck
(e) Knowledge Product (Non-material product such as a compound library, database, something that improves clinical practice, education, etc.)
(f) Other - Please Specify:

6. How does your candidate product aid the Warfighter, Veteran, Beneficiary, and/or General Population?

The proposed studies will aid the Warfighter, Veteran, Beneficiary and General Population who are unfortunately diagnosed with epithelial ovarian cancer in a number of ways. The studies will provided scientific rationale for developing urgently needed novel therapeutic strategies for ARID1A-mutated ovarian cancer, a disease that currently has no effective therapy. In the immediate short-term, the proposed studies provided fundamental mechanistic insights into the role of ARID1A mutation and HDAC6 inhibition in regulating the tumor immune microenvironment in ovarian cancer. In the long-term, they laid the critical foundation for developing a novel combinatory intervention strategy for ARID1A-mutated ovarian cancer.

7. Therapy / Product Development, Transition Strategies, and Intellectual Property

Describe the steps and relevant strategies required to move the candidate product (knowledge or tangible) to the next phase of development and/or commercialization. Please address any issues with intellectual property.

PIs are encouraged to explore the technical requirements and the current regulatory strategies involved in product development as well as to work with their organization's Technology Transfer Office (or equivalent regulatory/legal office), federal/international regulatory experts, to develop the transition plan and to explore developing relationships with industry, DoD advanced developers (e.g. USAMMDA), and/or other funding agencies to facilitate moving the product into the next phase.

HDAC6 inhibitor ACY1215 is now in clinical development for other cancer types, and anti-PD-L1 is FDA-approved. Thus, they are readily available for novel clinical application in ARID1A-mutated ovarian cancer. The challenge has to do with finding the right partners to move the discovery to clinical trials.

9. APPENDICES:

References

1. Trizzino, M., et al., *The Tumor Suppressor ARID1A Controls Global Transcription via Pausing of RNA Polymerase II.* Cell Rep, 2018. **23**(13): p. 3933-3945.
2. Zhu, H., et al., *BET Bromodomain Inhibition Promotes Anti-tumor Immunity by Suppressing PD-L1 Expression.* Cell Rep, 2016. **16**(11): p. 2829-2837.
3. Helming, K.C., et al., *ARID1B is a specific vulnerability in ARID1A-mutant cancers.* Nat Med, 2014. **20**(3): p. 251-4.
4. Pardoll, D.M., *The blockade of immune checkpoints in cancer immunotherapy.* Nat Rev Cancer, 2012. **12**(4): p. 252-64.
5. Adeegbe, D.O., et al., *Synergistic Immunostimulatory Effects and Therapeutic Benefit of Combined Histone Deacetylase and Bromodomain Inhibition in Non-Small Cell Lung Cancer.* Cancer Discov, 2017. **7**(8): p. 852-867.
6. Woan, K.V., et al., *Targeting histone deacetylase 6 mediates a dual anti-melanoma effect: Enhanced antitumor immunity and impaired cell proliferation.* Mol Oncol, 2015. **9**(7): p. 1447-1457.
7. Liu, Y., et al., *NK Cells Mediate Synergistic Antitumor Effects of Combined Inhibition of HDAC6 and BET in a SCLC Preclinical Model.* Cancer Res, 2018. **78**(13): p. 3709-3717.
8. Bitler, B.G., et al., *ARID1A-mutated ovarian cancers depend on HDAC6 activity.* Nat Cell Biol, 2017. **19**(8): p. 962-973.
9. Chandler, R.L., et al., *Coexistent ARID1A-PIK3CA mutations promote ovarian clear-cell tumorigenesis through pro-tumorigenic inflammatory cytokine signalling.* Nat Commun, 2015. **6**: p. 6118.
10. Shen, J., et al., *ARID1A deficiency promotes mutability and potentiates therapeutic antitumor immunity unleashed by immune checkpoint blockade.* Nat Med, 2018.
11. Binnewies, M., et al., *Understanding the tumor immune microenvironment (TIME) for effective therapy.* Nat Med, 2018.