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CONTRACTING ORGANIZATION: The Wistar Institute, Philadelphia, PA

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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.

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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.

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

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 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 year 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.

2) specific objectives;

The major objectives in the first year 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 micronenvironment 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.

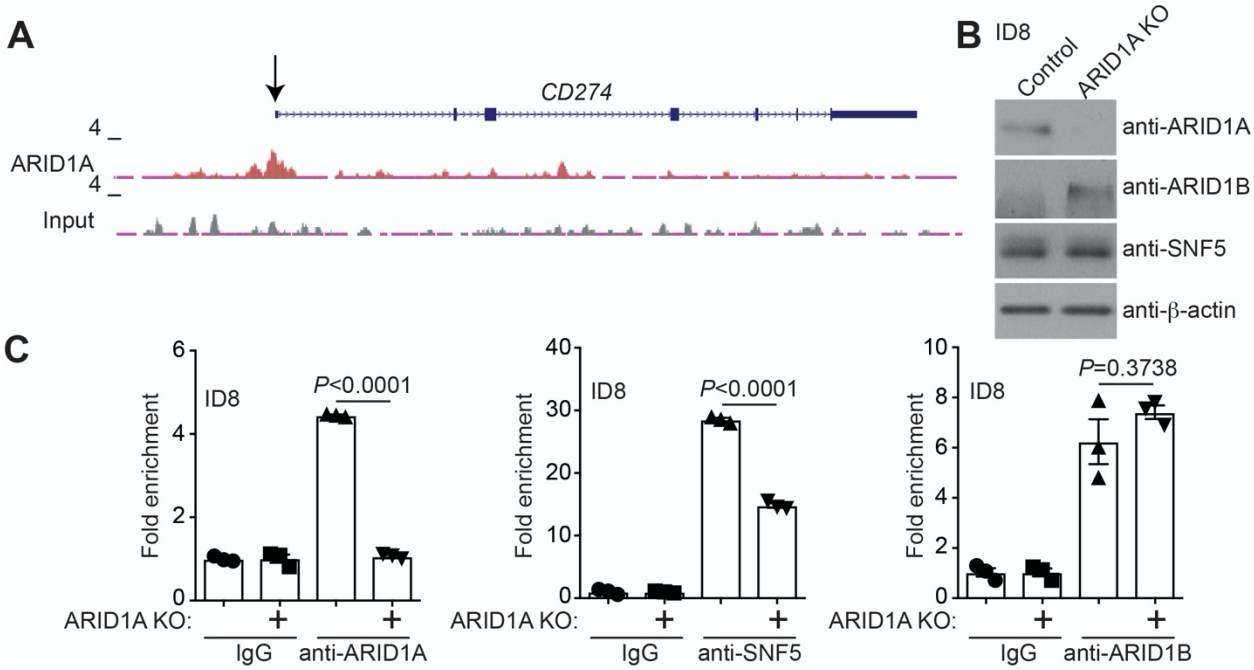

Figure 1. *CD274* is a direct ARID1A target gene in mouse ovarian cancer cells.
(**A**) ARID1A ChIP-seq track on the *CD274* gene locus in *ARID1A* wildtype RMG1 cells. (**B**) Expression of ARID1A, ARID1B, SNF5 and □-actin in the *ARID1A* wildtype control and ARID1A knockout mouse ovarian ID8-*Defb29/Vegf* 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.

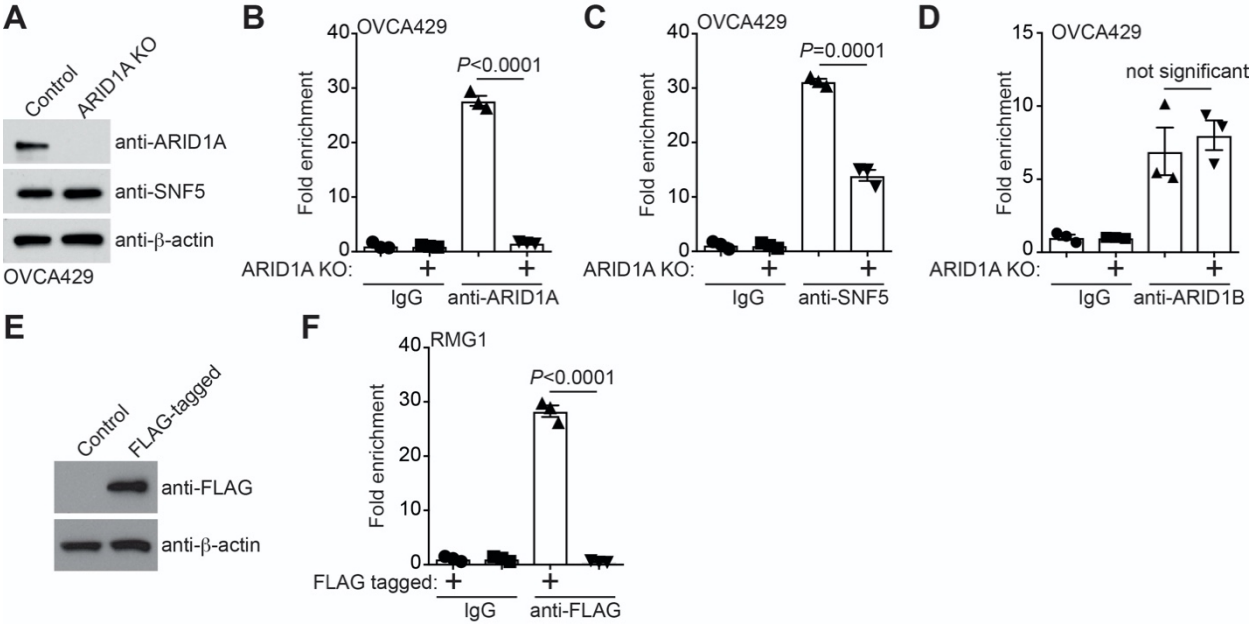

Figure 2. *CD274* is a direct ARID1A target gene in human ovarian clear cell ovarian cancer cells.

(**A**) Expression of ARID1A and SNF5 in the *ARID1A* wildtype control and ARID1A knockout human OVCA429 OCCC cells. □-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 (IFN□) plays a major role in inducing PD-L1 expression [4]. Thus, we examined the effects of ARID1A knockout on IFN□-induced PD-L1 expression. ARID1A knockout significantly enhanced the upregulation of *Cd274* mRNA and PD-L1 expression induced by IFN□ 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 IFN□ stimulation (**Figure 3B**). Together, we conclude that ARID1A represses *CD274* gene transcription at both the basal levels and in response to IFN□ stimulation.

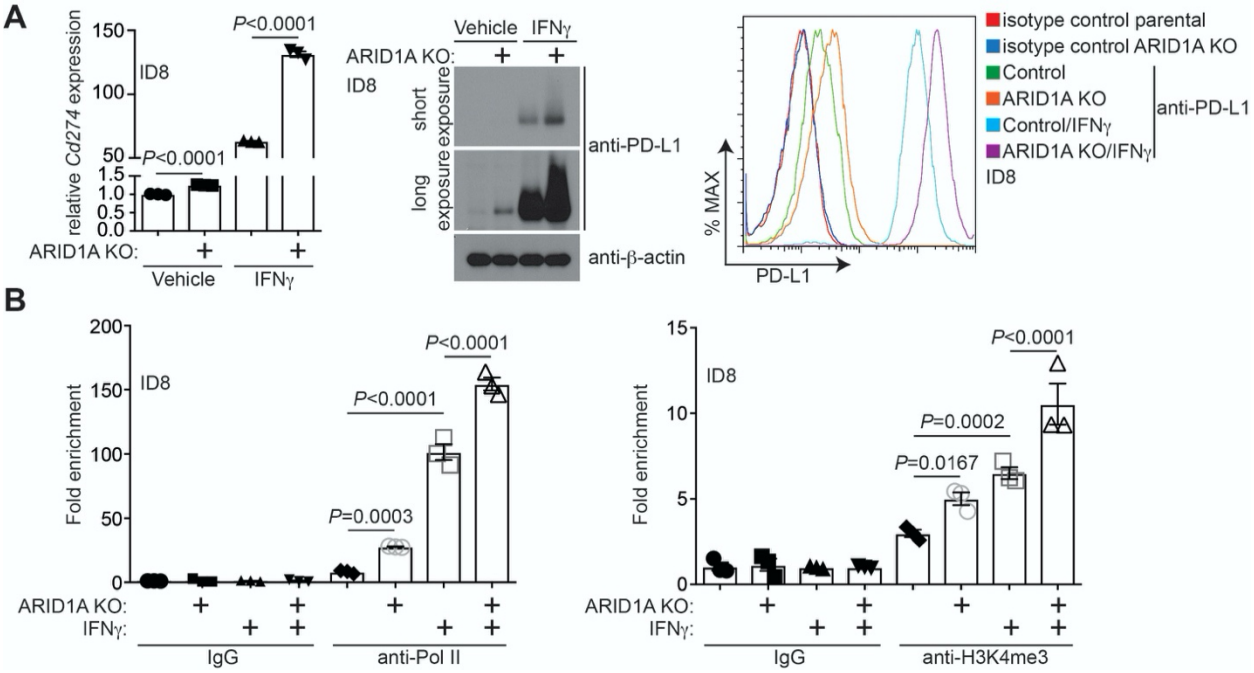

Figure 3. ARID1A transcriptionally represses *Cd274* in mouse ovarian cancer cells. (**A**) Expression of *Cd274* mRNA and PD-L1 protein in *ARID1A* wildtype control and ARID1A knockout mouse ovarian ID8-*Defb29/Vegf* cells treated with or without 20 ng/ml IFN□ determined by qRT-PCR (left) or immunoblot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

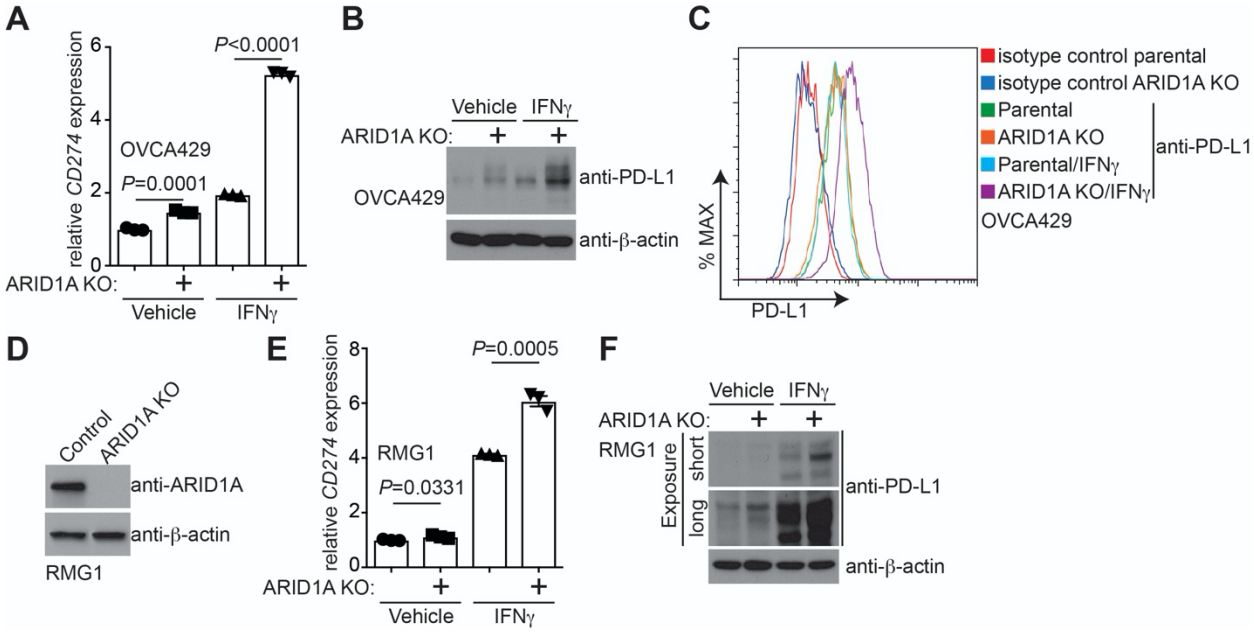

Figure 4. ARID1A represses *CD274* transcription in human ovarian clear cell cancer cells. (**A-B**) Expression of *CD274* mRNA (A) and PD-L1 protein (B) in *ARID1A* wildtype control and ARID1A knockout OVCA429 OCCC cells treated with or without 20 ng/ml IFN□ determined by qRT-PCR or immunoblot, respectively. (**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, IFN□$^{+}$ 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.

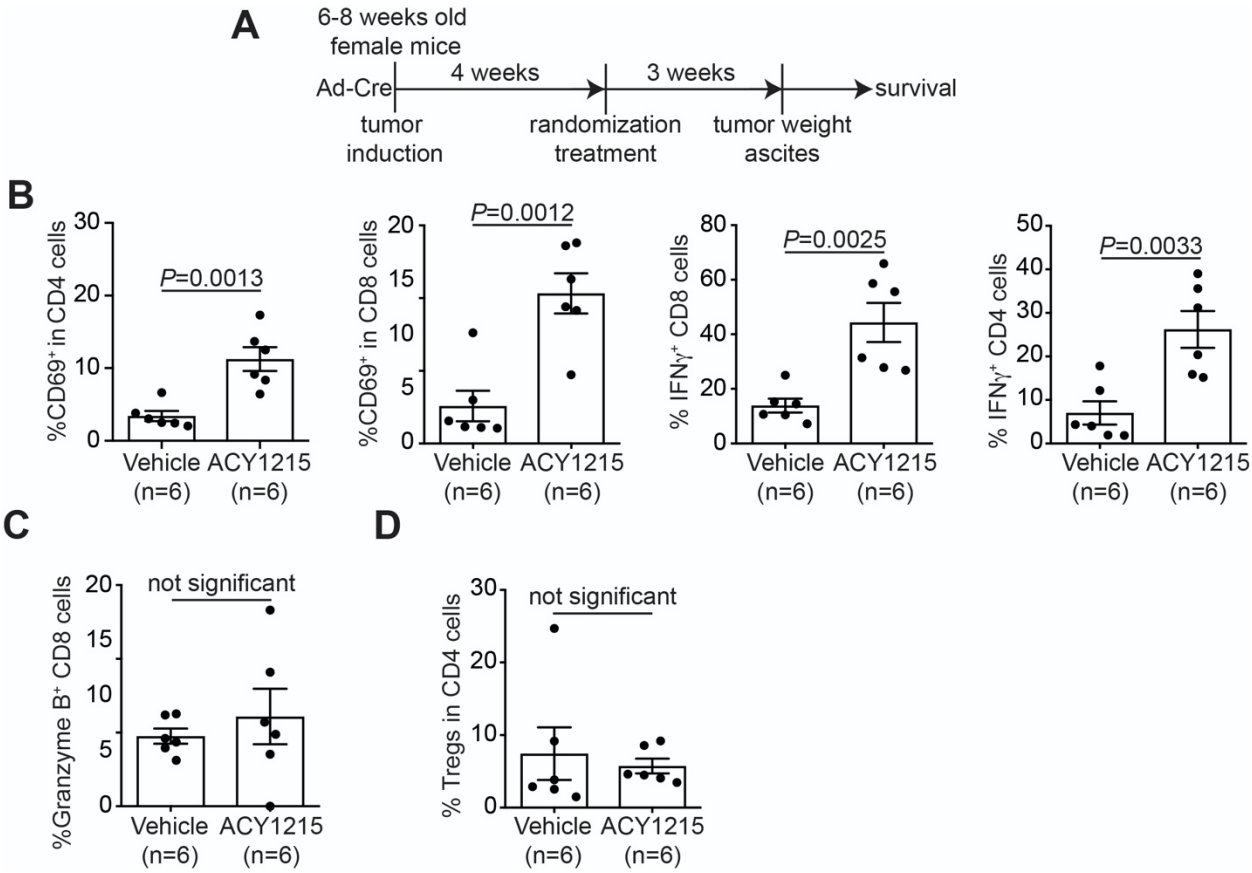

Figure 5. ACY1215 boosts antitumor immunity.
ARID1A$^{flox/flox}$*/PIK3CA*^{H1047R} OCCCs were induced by intrabursal adenovirus-encoded Cre injection and allowed to establish for four weeks (**A**). The mice were randomized to treat with vehicle control or ACY1215 (50 mg/kg daily by i.p.) for an additional 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.

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 perform functional studies to characterize the identified changes in the immune modulating cells.
2. To breed and characterize the conditional genetic ovarian clear cell cancer mouse models for the proposed combination of HDAC6 inhibition and anti-PD-L1 antibody combination.

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

1. **Journal publications.** Wu S, Fukumoto T, Lin J, Nacarelli T, Wang Y, Ong D, 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**. Therapeuitc targeting glutamine dependence in SWI/SNF-inactivated cancers. ***Nature Cancer***, accepted.

2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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):	*N/A*
Nearest person month worked:	*1 cm*
Contribution to Project:	Supervised the study.
Funding Support:	This award

Name:	Takeshi Fukumoto
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1 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:	*2 cm*
Contribution to Project:	Performed the study.
Funding Support:	This award

Name:	Pingyu Liu
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*5 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:

Termination of "Identification of Ovarian Cancer Plasma Biomarkers"
Termination of "Synthetic lethal therapeutic approaches for ARID1A-mutated ovarian cancer"
Termination of "Mechanism of myeloid cell defect in cancer"
Activation of "Synthetic lethality based combination approaches to ARID1A mutation in ovarian cancer"
Activation of "Regulation of tumor recurrence by stress activated neutrophils"

Dr. Qin Liu:

Termination of "Identification of Ovarian Cancer Plasma Biomarkers"
Termination of "The Effect of Binding of fH to Meningococcal fHbp Vaccine on Antibody Protection"
Termination of "HSP70 and Melanoma"
Termination of "p53 Variants in Cancer Risk and Therapy"
Termination of "Functional Analysis of p53 Polymorphic Variants"
Termination of "Synthetic Lethal Therapeutic Approaches for ARID1A-mutated Ovarian Cancer"
Termination of "Effects of the Aged Microenvironment on Tumor Dormancy"
Termination of "Promotion of tumor invasion and pseudosenescence by the aging microenvironment"
Termination of "Defining Carcinoma-Astrocyte Interactions in Breast Cancer Brain Metastasis"
Termination of "A plasticity and reprogramming paradigm for therapy resistance at the single cell level"
Termination of "The role of p53 in Iron Overload"
Termination of "Glycan-lectin Interactions in the Development of Non-AIDS-defining Malignancies"
Activation of "Role of an Integrator-EGR axis in the regulation of myeloid enhancers"
Activation of "Host Glycomic Determinants of HIV Persistence in vivo"
Activation of "Sialic Acid Modulation of HIV-associated Chronic Inflammaging"
Activation of "Synthetic lethality-based combination approaches to ARID1A mutation in ovarian cancer"
Activation of "Effects of Mu-opiate receptor engagement on the persistence of HIV-associated activation and viral reservoirs in individuals receiving medication assisted treatment for opioid use disorder"
Activation of "Immunological approaches for ARID1A-mutated ovarian cancer" (Note: This is the grant that you are reporting. I usually do not list it as a change when I prepare RPPRs. But, I am listing it here in case DoD's reporting criteria is different.)

Activation of "Understanding PPARgamma signaling in melanoma brain metastasis"
Activation of "Understanding and Overcoming Resistance to BRAF/MEK Kinase Inhibitors in Melanoma"
Activation of "Development of Novel Small-Molecule Rb protein modulator for Ovarian Cancer Immunotherapy"
Activation of "A cancer-derived truncating mutation in disease penetrance and progression of MSI CRC"
Activation of "Development and pre-clinical testing of a prophylactic SARS-CoV-2 vaccine"

What other organizations were involved as partners?
Nothing to report

8. SPECIAL REPORTING REQUIREMENTS
Nothing to report

9. APPENDICES:
Nothing to report