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PRINCIPAL INVESTIGATOR: Qin Liu

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Qin Liu (PI), Rugang Zhang (Co-Investigator)

E-Mail: qliu@wistar.org, RZhang11@mdanderson.org

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The Wistar Institute (PI),
AND ADDRESS(ES)
3601 Spruce Street
Philadelphia, PA 19104

The University of Texas M. D. Anderson
Cancer Center (Co-Investigator)
1515 Holcombe Boulevard
Huston, TX 21702

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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. Emerging evidence supports the idea that the SWI/SNF complexes play a critical role in the tumor metabolism. Our preliminary data show that ARID1A inactivation decreases glucose uptake and creates a dependence on the glutamine metabolism. Indeed, ARID1A inactivation sensitizes ovarian cancer to glutaminase (GLS) inhibition using a clinically applicable GLS inhibitor such as CB-839. In addition, there is evidence to support that the SWI/SNF complex inactivation renders tumors susceptible to checkpoint inhibition. Our preliminary data show that ARID1A mutation sensitizes ovarian cancer to anti-PD-L1 treatment. This is consistent with a recent publication showing that ARID1A mutation increases the PD-L1 expression in cancer cells. Notably, the competition for glutamine in the tumor microenvironment can suppress T cell proliferation. Consistently, the inhibition of GLS activity in tumor cells potentiate T cell proliferation without suppressing T cell function. Together, these findings raised the possibility that the inhibition of GLS activity in ARID1A-mutated ovarian cancer will not only suppresses the growth of ARID1A mutant cancer cells but also potentiate T cell proliferation and thus enhance checkpoint blockade therapy. Our **central hypothesis** is that ARID1A-mutated ovarian cancer can be therapeutically eradicated by the combination of a clinically applicable glutaminase inhibitor such as CB-839 and anti-PD-L1 immune checkpoint blockade.

15. SUBJECT TERMS
Epithelial ovarian cancer, ovarian clear cell carcinoma, ARID1A, SWI/SNF, glutaminase, 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. Emerging evidence supports the idea that the SWI/SNF complexes play a critical role in the tumor metabolism. Our preliminary data show that ARID1A inactivation decreases glucose uptake and creates a dependence on the glutamine metabolism. Indeed, ARID1A inactivation sensitizes ovarian cancer to glutaminase (GLS) inhibition using a clinically applicable GLS inhibitor such as CB-839. In addition, there is evidence to support that the SWI/SNF complex inactivation renders tumors susceptible to checkpoint inhibition. Our preliminary data show that ARID1A mutation sensitizes ovarian cancer to anti-PD-L1 treatment. This is consistent with a recent publication showing that ARID1A mutation increases the PD-L1 expression in cancer cells. Notably, the competition for glutamine in the tumor microenvironment can suppress T cell proliferation. Consistently, the inhibition of GLS activity in tumor cells potentiate T cell proliferation without suppressing T cell function. Together, these findings raised the possibility that the inhibition of GLS activity in ARID1A-mutated ovarian cancer will not only suppresses the growth of ARID1A mutant cancer cells but also potentiate T cell proliferation and thus enhance checkpoint blockade therapy. Our **central hypothesis** is that ARID1A-mutated ovarian cancer can be therapeutically eradicated by the combination of a clinically applicable glutaminase inhibitor such as CB-839 and anti-PD-L1 immune checkpoint blockade.

KEYWORDS:

Epithelial ovarian cancer, ovarian clear cell carcinoma, ARID1A, SWI/SNF, glutaminase, 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 clinically applicable glutaminase inhibitor CB-839 and immune checkpoint anti-PD-L1.

Specific Aim 1: To investigate the mechanism underlying the dependence of ARID1A mutation on glutamine metabolism.

Specific Aim 2: To develop a novel therapeutic approach for ARID1A-mutated ovarian cancer by combining a clinically applicable glutaminase inhibitor 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 investigated the mechanism by which ARID1A regulates glutaminase expression and activity.
2. We determined whether the observed glutamine dependence is ARID1A status dependent.
3. We determined ARID1A inactivation creates a dependence on GLS1 enzymatic activity.

4. We determined the glutamine dependence created by ARID1A inactivation is mediated by the increase in aspartate.

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

1. To elucidate how ARID1A may regulate expression of GLS at the transcriptional level.
2. To determine the effects of ARID1A status on sensitivity to glutamine starvation.
3. To determine whether ARID1A inactivation creates a dependence on GLS1 enzymatic activity.
4. To determine whether the glutamine dependence created by ARID1A inactivation is mediated by the increase in aspartate.

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

ARID1A inactivation creates a dependence on glutamine

To explore the potential role of ARID1A in glutamine dependence, we knocked out ARID1A in ARID1A wildtype RMG1 OCCC cells to mimic loss of ARID1A protein expression caused by >90% of ARID1A mutations (**Fig. 1A**). Notably, ARID1A knockout does not affect cell growth rates [1]. Compared with ARID1A wildtype cells, ARID1A knockout cells significantly exacerbated the growth suppression induced by glutamine deprivation (**Fig. 1B-C**). Similar observations were made in additional isogenic ARID1A wildtype and knockout OCCC cell lines (**Fig. 2**). Notably, glucose uptake was decreased by ARID1A knockout (**Fig. 3A-B**), which correlates with a decrease in sensitivity to glucose deprivation (**Fig. 3C**). These results indicate that ARID1A inactivation creates a dependence on glutamine.

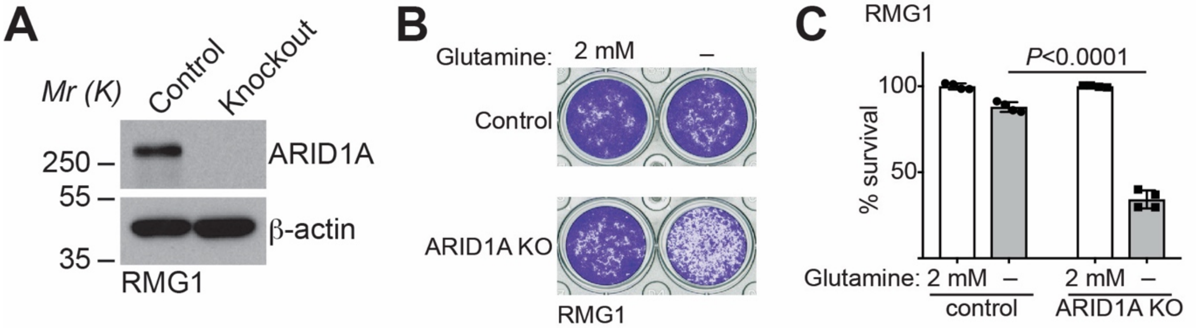

Fig. 1: ARID1A inactivation creates a dependence on glutamine in OCCC RMG1 cells. **A,** Validation of ARID1A knockout in parental and ARID1A knockout RMG1cells. **B-C,** Colony formation (**B**) and quantification (**C**) of parental and ARID1A knockout RMG1 cells cultured in medium with or without glutamine deprivation for 12 days. Shown are representative of four independent experiments with similar results in **B**. n= 4 independent experiments in **C**. Error bars represent mean with s.d. P values were calculated using two-tailed Student t-test.

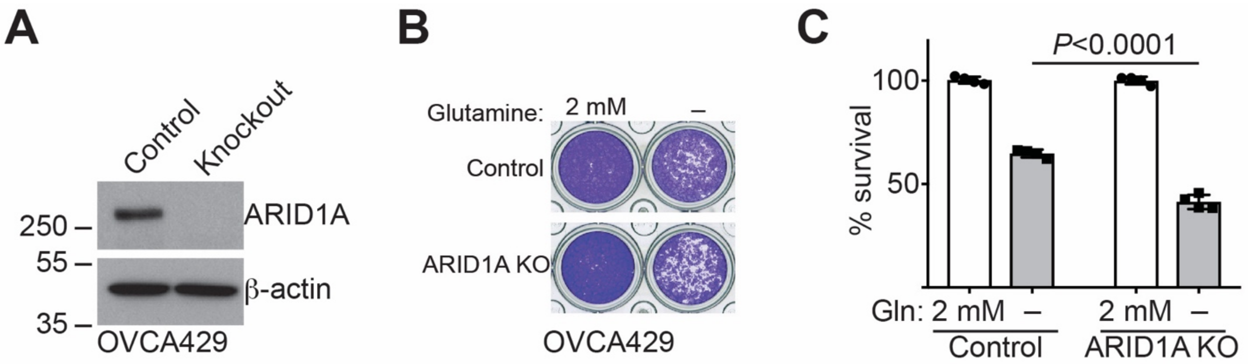

Fig. 2: ARID1A inactivation creates a dependence on glutamine in OCCC OVCA429 cells. **A,** Validation of ARID1A knockout in parental and ARID1A knockout OVCA429 cells. **B-C,** Colony formation (**B**) and quantification (**C**) of parental and ARID1A knockout OVCA429 cells cultured in medium with or without glutamine deprivation for 12 days. Shown are representative of four independent experiments with similar results in **B**. n= 4 independent experiments in **C**. Error bars represent mean with s.d. P values were calculated using two-tailed Student t-test.

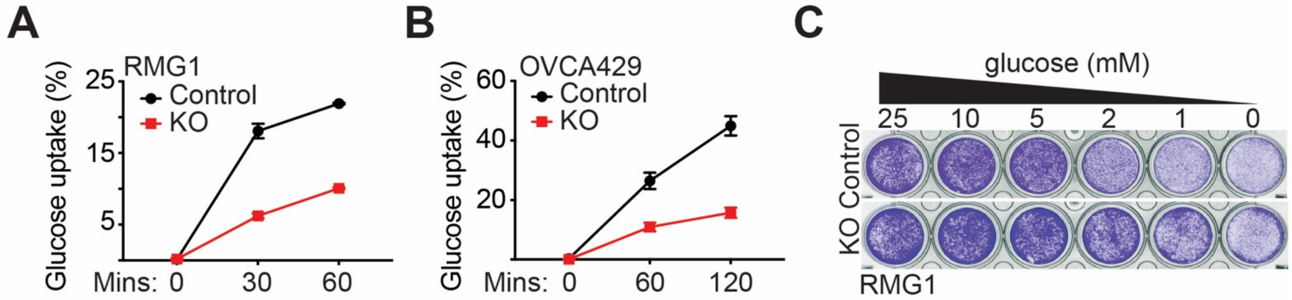

Fig. 3: ARID1A inactivation decreases sensitive of OCCC cells to glucose deprivation. **A-B,** A fluorescence glucose analog 2-NBDG-based glucose uptake assayed by flow cytometry analysis for the indicated parental and ARID1A knockout RMG1 (**A**) or OVCA429 (**B**) cells. **C,** Colony formation of parental and ARID1A knockout RMG1 cells cultured in the medium with indicated concentration of glucose for 12 days. n= 4 technical replicates. Error bars represent mean with s.d. P values were calculated using two-tailed t-test.

Inactivation of SWI/SNF complex increases GLS1 expression

We next sought to determine the mechanism underlying the observed glutamine dependence by ARID1A inactivation. Toward this goal, we cross-referenced ARID1A chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) analysis in ARID1A wildtype RMG1 cells with differentially expressed genes based on RNA-seq analysis in ARID1A wildtype control and knockout RMG1 cells. The analysis revealed GLS1 as the top direct ARID1A target gene that was significantly upregulated by ARID1A knockout in glutamine metabolic pathway (**Fig. 4A-B**), suggesting that ARID1A functions as a transcriptional repressor of GLS1. Consistently, GLS1 is also a target of SNF5, a core subunit of the SWI/SNF complex and ARID1A knockout increased the association of RNA polymerase II (Pol II)'s association with the GLS1 promoter in RMG1 cells (**Fig. 4C**).

We validated the upregulation of GLS1 expression and the increase in glutaminase activity in ARID1A knockout RMG1 cells (**Fig. 5A-B**). Notably, the observed increase in both GLS1 expression and glutaminase activity was rescued by restoration of ARID1A expression, indicating these are ARID1A expression dependent instead of potential off-target effects (**Fig. 5A-B**). In addition, ARID1A and other core subunits of the SWI/SNF complex such as BAF155 and SNF5 directly bound to the GLS1 promoter as determined by cut-run or ChIP-qPCR analysis (**Fig. 5C**). Notably, the association of BAF155 and SNF5 with the GLS1 promoter was reduced by ARID1A knockout (**Fig. 5C**), suggesting that the observed repression of GLS1 by ARID1A is SWI/SNF complex dependent. The enhanced association of RNA Pol II with the GLS1 promoter by ARID1A knockout was also validated (**Fig. 5C**). Similar findings were made in additional ARID1A wildtype and knockdown or knockout isogenic cell lines (**Fig. 5D-F**). Conversely, restoration of wildtype ARID1A expression in ARID1A-mutated cells suppressed GLS1 expression and reduced glutaminase activity (**Fig. 6**). Together, we conclude that ARID1A-containing SWI/SNF complex functions as a repressor of GLS1 expression.

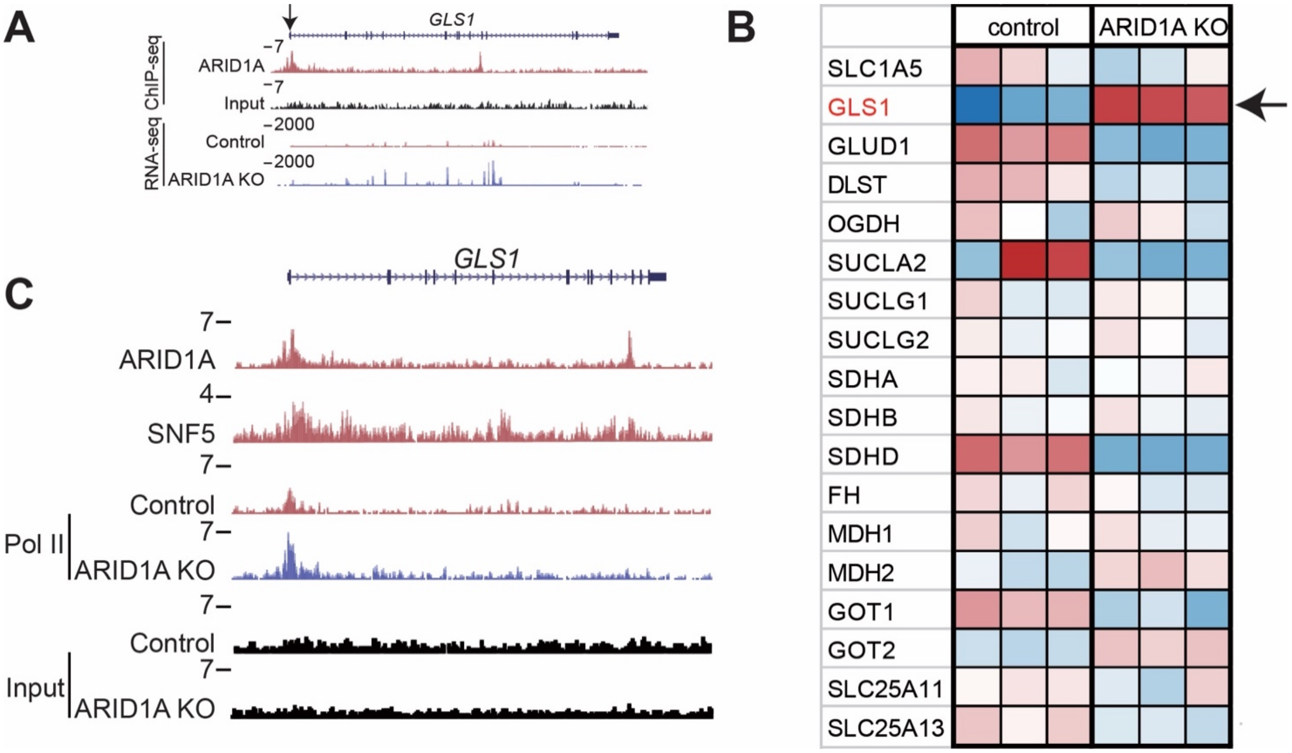

Fig. 4: GLS1 is a direct target of the SWI/SNF complex.
A, ARID1A ChIP-seq and input tracks and RNA-seq tracks in the GLS1 gene locus in parental and ARID1A knockout RMG1 cells. Shown are representative of three independent experiments with similar results. **B,** Expression of glutamine metabolism related genes in control and ARID1A knockout RMG1 cells determined by RNA-seq analysis. Note that GLS1 shows the highest upregulation in response to ARID1A knockout. **C,** The indicated ChIP-seq and input tracks in the GLS1 gene locus in parental and ARID1A knockout RMG1 cells [2].

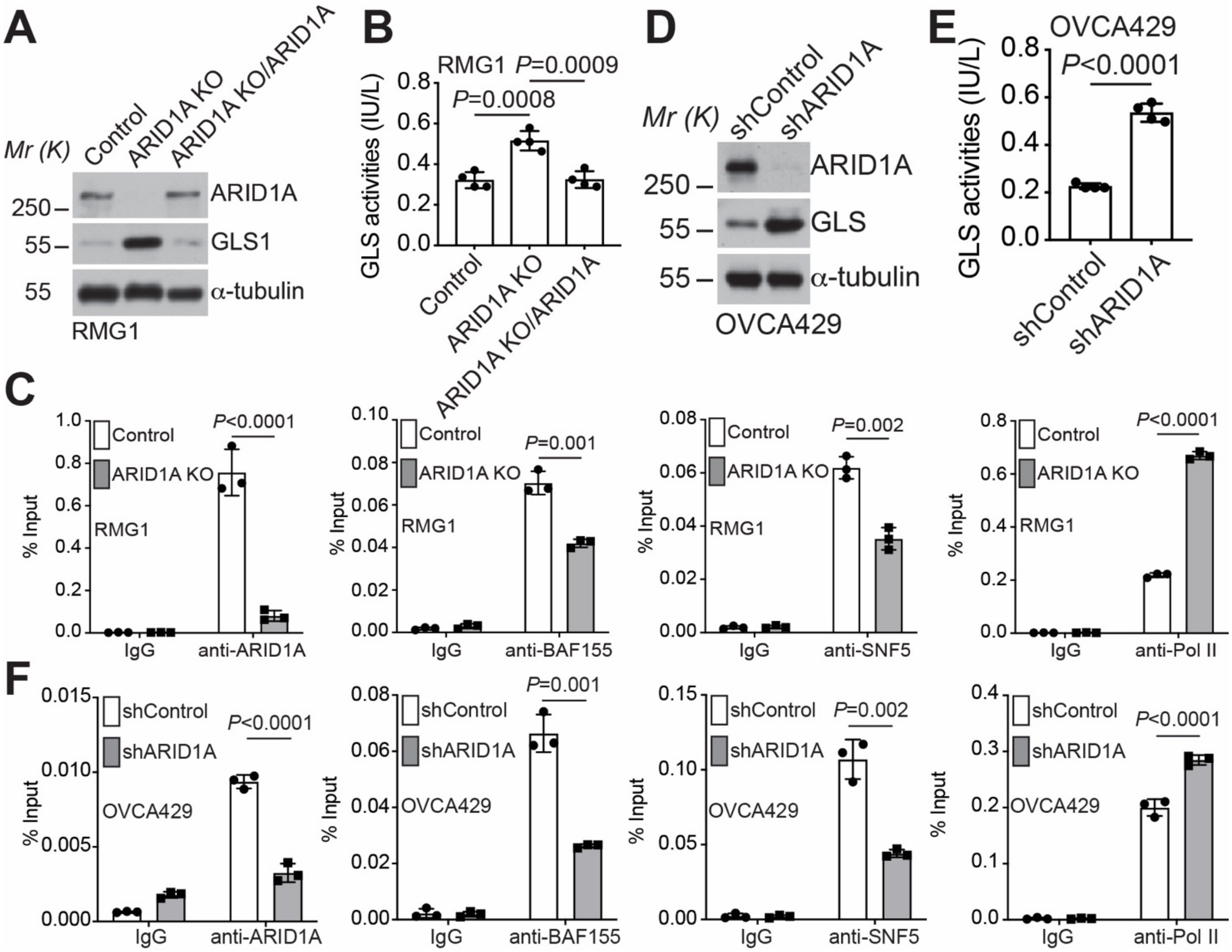

Fig. 5: Validation of GLS1 is a direct target of the SWI/SNF complex.

A-B, Parental and ARID1A knockout RMG1 cells with or without wildtype ARID1A restoration were examined for expression of ARID1A and GLS1 by immunoblot (**A**) or measured for glutaminase activity (**B**). n= 4 independent experiments. **C,** The association of ARID1A, BAF155, SNF5 and RNA Pol II with the GLS1 gene promoter in the indicated cells was examined by ChIP-qPCR analysis. An isotype matched IgG was used as a negative control. n= 3 independent experiments. **D-E,** Control and ARID1A knockdown OVCA429 cells were examined for expression of ARID1A and GLS1 by immunoblot (**D**) or measured for glutaminase activity (**E**). n= 4 technical replicates. **F,** The association of ARID1A, BAF155, SNF5 and RNA Pol II with the GLS1 gene promoter in parental and ARID1A knockdown OVCA429 cells was examined by ChIP-qPCR analysis. An isotype matched IgG was used as a control. n = 3 technical replicates. Error bars represent mean with s.d.. P values were calculated using two-tailed Student t-test.

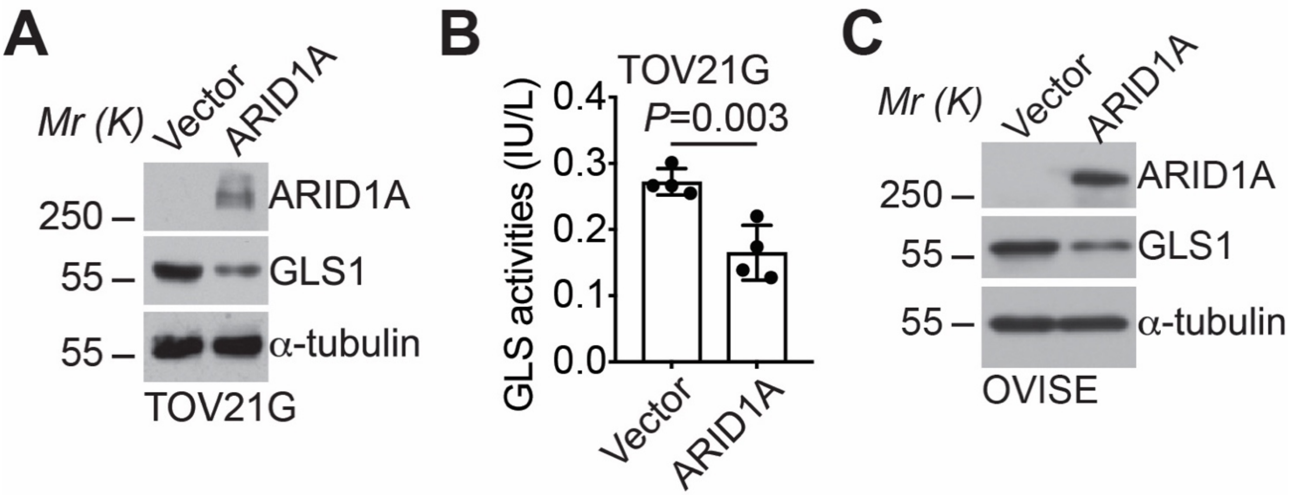

Fig. 6: Restoration of wildtype ARID1A expression in ARID1A mutant OCCC cells suppresses GLS1 expression.
A-B, ARID1A-mutated TOV21G cells with or without wildtype ARID1A restoration were examined for ARID1A and GLS1 expression by immunoblot (**A**) or measured for glutaminase activity (**B**). n= 4 independent experiments. **C,** Control and wildtype ARID1A ectopically expressing OVISE cells were examined for expression of ARID1A and GLS1 by immunoblot. Error bars represent mean with s.d. P values were calculated using two-tailed Student t-test.

ARID1A inactivation sensitizes cells to GLS inhibition

Since ARID1A inactivation creates glutamine dependence and upregulates GLS1 expression, we next sought to determine whether ARID1A inactivation sensitizes cells to GLS1 inhibition. Toward this goal, we inhibited GLS1 enzymatic activity both genetically by shRNA-mediated knockdown and using a small molecule inhibitor of glutaminase activity. Indeed, genetically knocking down GLS1 expression was significantly more effective in suppressing the growth of ARID1A knockout cells compared to controls (**Fig. 7A-B**). We also tested CB-839, a specific glutaminase inhibitor [3], in *ARID1A* wildtype control and knockout RMG1 cells. We chose CB-839 for our experiments because it is the only GLS inhibitor that is now in clinical trials for other diseases and is proven safe in clinical trials including in combination studies [3, 4]. Indeed, compared with *ARID1A* wildtype control cells, ARID1A knockout significantly decreased the IC_{50} of CB-839 in RMG1 cells by more than 300-fold (**Fig. 7C**). The observed effects are ARID1A dependent because the decrease in CB-839 IC_{50} can be rescued by ectopic expression of wildtype ARID1A (**Fig. 7C**). Notably, restoration of ARID1A expression in *ARID1A*-mutated cells significantly reduced the sensitivity to CB-839 (**Fig. 7D**). Likewise, CB-839 was effective in suppressing the growth of ARID1A or SMARCA4 inactivated primary OCCC cultures (**Fig. 7E-F**). Together, we conclude that inactivation of the SWI/SNF complex sensitizes cells to GLS1 inhibition.

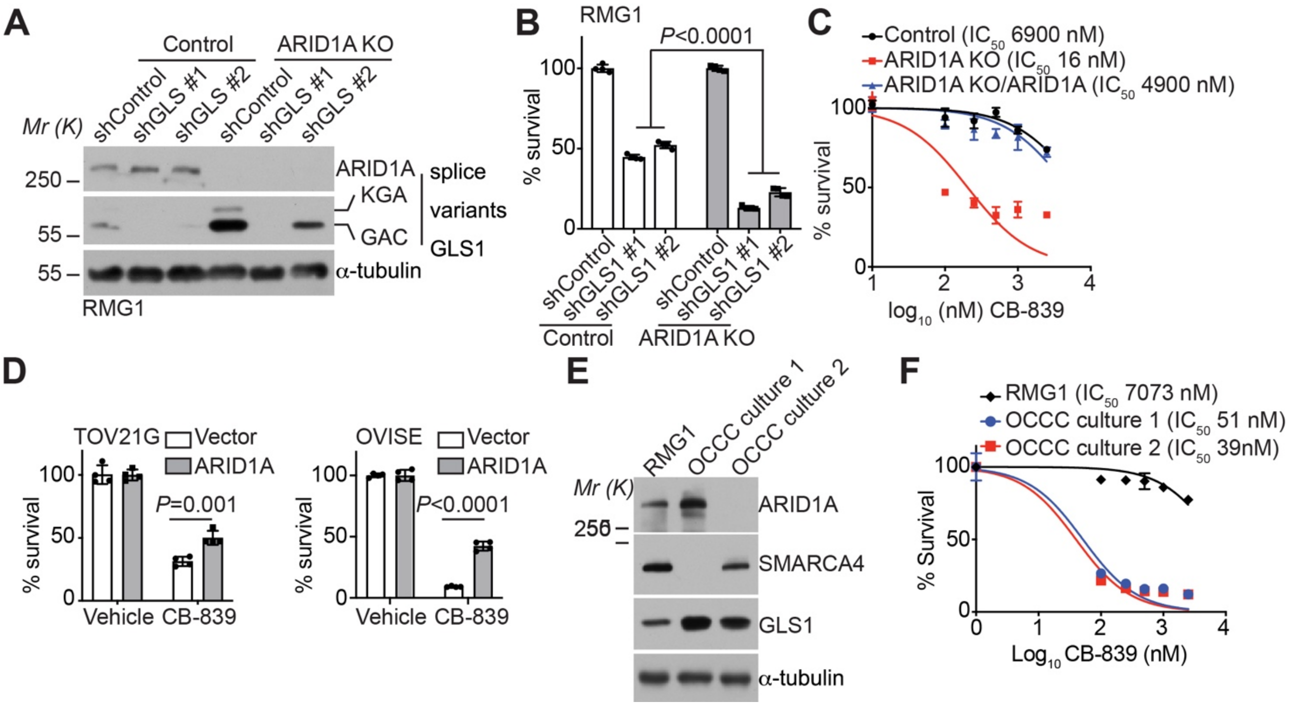

Fig. 7: Inactivation of the SWI/SNF complex sensitizes cells to glutaminase inhibition. **A-B,** Parental and ARID1A knockout RMG1 cells expressing the indicated shGLS1s or control were examined for ARID1A and GLS1 expression by immunoblot (**A**) or subjected to colony formation and the colonies formed by the indicated cells were qualified (**B**). **C**, Dose response curves of the indicated parental and ARID1A knockout RMG1 cells with or without wildtype ARID1A restoration to glutaminase inhibitor CB-839 determined by colony formation assay. **D,** Quantification of growth of TOV21G and OVISE cells with or without wildtype ARID1A restoration treated with or without 0.05 μM or 10 μM CB-839 for 12 days based on colony formation assay. **E**, Expression of ARID1A and GLS1 in the indicated ARID1A or SMARCA4-inactivated primary OCCC cultures. **F,** Dose response curves of the indicated ARID1A or SMARCA4-inactivated primary OCCC cultures to glutaminase inhibitor CB-839 determined by colony formation assay. RMG1 cells were used as a control. Error bars represent mean with s.d. from 4 technical replicates. All data were representative of three independent experiments. *P* values were calculated using two-tailed *t*-test.

ARID1A inactivation increases glutamine utilization and metabolism through the TCA cycle

We next sought to determine how the ARID1A status differentially affects glutamine utilization. Toward this goal, we performed liquid chromatography and mass-spectrometry (LC-MS)/MS based analysis of metabolites in *ARID1A* wildtype control and knockout RMG1 OCCC cells with or without GLS inhibition by CB-839. Metabolic profiling revealed that ARID1A inactivation increases glutamine utilization by the TCA cycle and the use of glutamine to support aspartate and nucleotide biosynthesis (**Fig. 8A**). Pathway analysis revealed malate-aspartate shuttle as the top pathway enriched based on the differential response to CB-839 between ARID1A knockout cells and controls (**Fig. 8B**). This further supports that ARID1A inactivation promotes aspartate and nucleotide synthesis from glutamine through the TCA cycle. Cells were next incubated with $^{13}C_5$-glutamine to infer glutamine metabolism and associated metabolic pathways. The $^{13}C_5$-glutamine stable isotope tracer analysis revealed that ARID1A knockout increased the metabolism through glutamate, TCA cycle metabolites (such as α-ketoglutarate and citrate), aspartate and nucleotides (such as UMP) (**Fig. 8C-D**). This suggests that in addition to increasing glutamine uptake by upregulating GLS1, ARID1A inactivation also increased the utilization of key glutamine metabolism metabolites such as aspartate to support the growth of ARID1A inactivated cells.

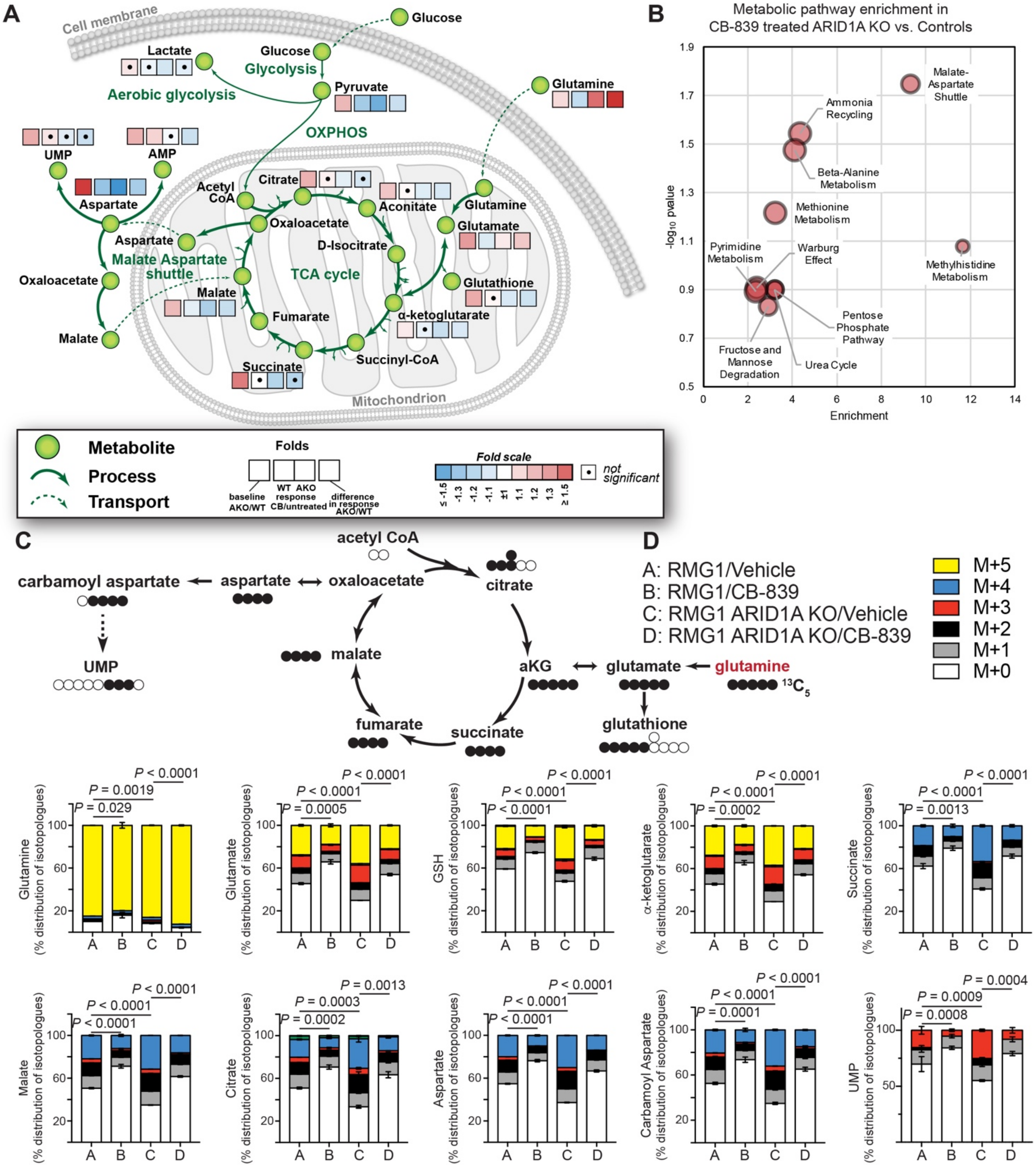

Fig. 8: ARID1A inactivation increases glutamine utilization and metabolism.
A, Control and ARDI1A knockout RMG1 cells treated with or without glutaminase inhibitor CB-839 were subjected to LC-MS/MS analysis. Heat map indicates fold changes in comparison to parental control without CB-839 treatment (n= 3 technical replicates). **B,** Analysis of metabolic pathways

enriched in CB-839 treated ARID1A knockout RMG1 cells compared with CB-839 treated parental RMG1 cells. **C,** Schematic of glutamine tracing of aspartate biogenesis. **D**, The indicated cells were incubated for 16 hours in the presence of $^{13}C_5$-glutamine and intracellular metabolites were extracted for analysis by LC-MC to evaluate glutamine-dependent metabolism. Mass isotopologues (M+*X*) analysis of the indicated metabolites are shown as percentage of indicated number of carbons labeled with heavy isotype (n= 3 technical replicates). Error bars represent mean with s.d. from 4 technical replicates unless otherwise stated. All data were representative of three independent experiments. *P* values were calculated using two-tailed *t*-test.

The dependence on GLS enzymatic activity created by ARID1A inactivation is mediated by aspartate metabolism

Notably, aspartate utilization was increased by ARID1A knockout as indicated by the increase in metabolites such as AMP, UMP and N-acetylaspartate (**Fig. 9A**). Consistent with these findings, the addition of aspartate in the culture medium of *ARID1A*-mutated or knockout cells reduced the sensitivity to CB-839 (**Fig. 9B-C**). In addition, ectopic expression of aspartate transporter SLC1A3 in RMG1 knockout cells that do not express endogenous SLC1A3 reduced the sensitivity to CB-839 (**Fig. 9D**), further supporting that the observed effects are due to changes in aspartate. Finally, supporting the notion that the increase in aspartate generated from glutamine in ARID1A-inactivated cells was utilized for nucleotide synthesis, CB-839 treatment significantly reduced S phase of the cell cycle where nucleotide is utilized for DNA replication (**Fig. 9E**). Together, we conclude that ARID1A inactivation creates glutamine dependence through both GLS1 upregulation and glutamine utilization such as aspartate generation and nucleotide synthesis.

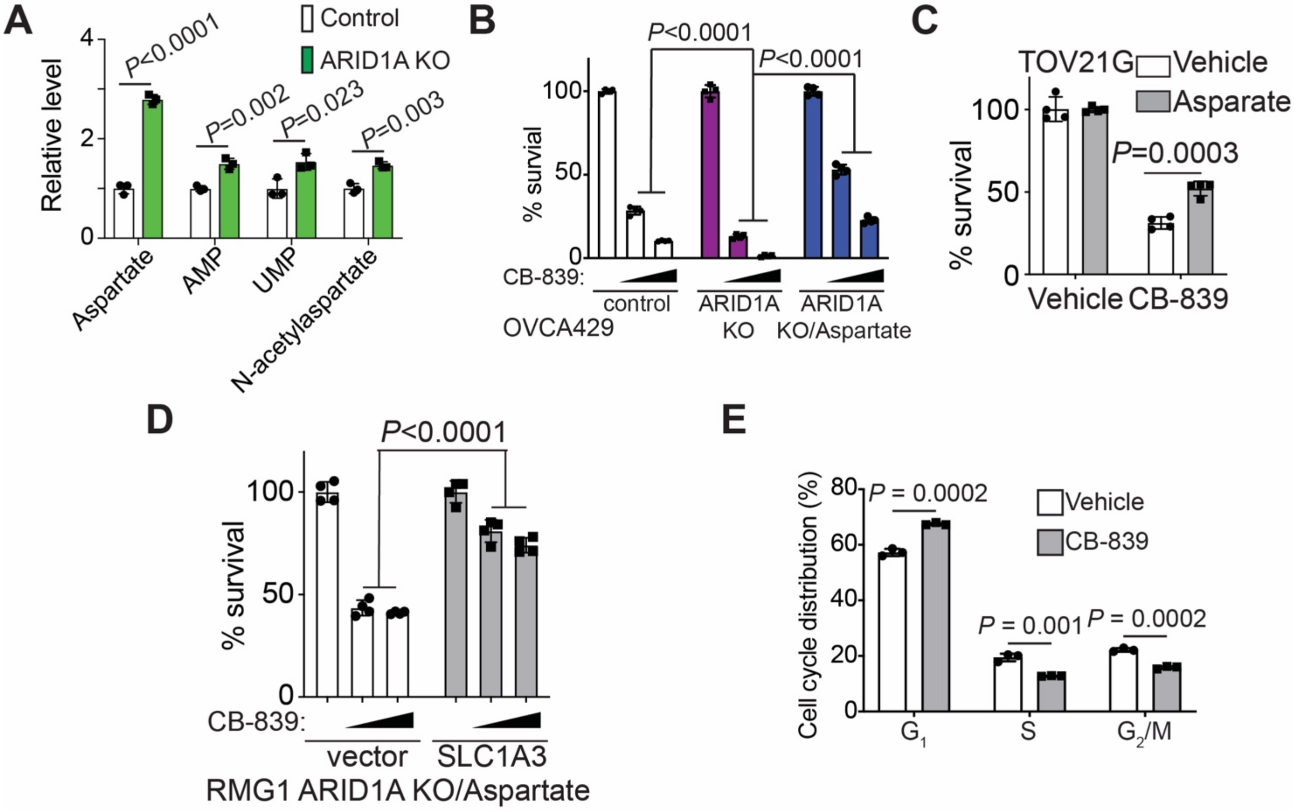

Fig. 9: ARID1A inactivation increases aspartate generation and nucleotide synthesis.
A, Quantification the indicated metabolites determined by glutamine tracing in control and ARID1A knockout RMG1 cells. n= 3 technical replicates. **B,** Quantification of colony formation by parental or ARID1A knockout OVCAR429 cells cultured in medium supplemented with or without 5 mM

aspartate treated with or without CB-839 (0.1 μM or 0.25 μM). n= 4 technical replicates. **C,** Quantification of colony formation of TOV21G cells treated with or without 0.05 μM CB-839 cultured in the medium supplemented with or without 5 mM aspartate. **D**, Quantification of colony formation by ARID1A knockout RMG1 cells with or without ectopic aspartate transport SLC1A3 expression cultured in medium supplemented with 5 mM aspartate treated with or without the indicated concentration of CB-839. . **E,** Cell cycle distribution in RMG1 ARID1A KO cells treated with or without 1 μM CB-839 for 72 hrs determined by flow cytometry analysis. n= 3 technical replicates. Error bars represent mean with s.d. from 4 technical replicates unless otherwise stated. All data were representative of three independent experiments. *P* values were calculated using two-tailed *t*-test.

Glutaminase inhibitor synergizes with immune checkpoint blockade in an ARID1A-inactivated immunocompetent OCCC model.

Lymphocyte function in the tumor microenvironment is regulated by glutamine metabolism [5]. Notably, in a conditional genetic *Arid1a*$^{flox/flox}$ */Pik3ca*^{H1047R} OCCC mouse model as we and others published [6, 7], CB-839 treatment significantly increased glutamine levels in the treated tumors (**Fig. 10A**). Notably, CB-839 treatment along significantly reduced the burden in the pre-established genetic OCCC model (**Fig. 10B**). *ARID1A* mutation confers sensitivity to immune checkpoint blockades such as anti-PDL1 [8, 9]. In addition, recent evidence shows that glutamine antagonism in effector T cells can be exploited as a “metabolic checkpoint” [10]. Thus, we examined whether CB-839 synergizes with anti-PDL1 in the *Arid1a/Pik3ca* immune competent OCCC genetic mouse model. Indeed, a combination of CB-839 and anti-PDL1 was significantly more effective in reducing the tumor burden of tumor-bearing mice compared with either one of the individual treatments (**Fig. 10C**). Together, we conclude that clinically applicable GLS inhibitor CB-839 synergizes with immune checkpoint blockade in suppressing the growth of ARID1A-inactivated OCCC.

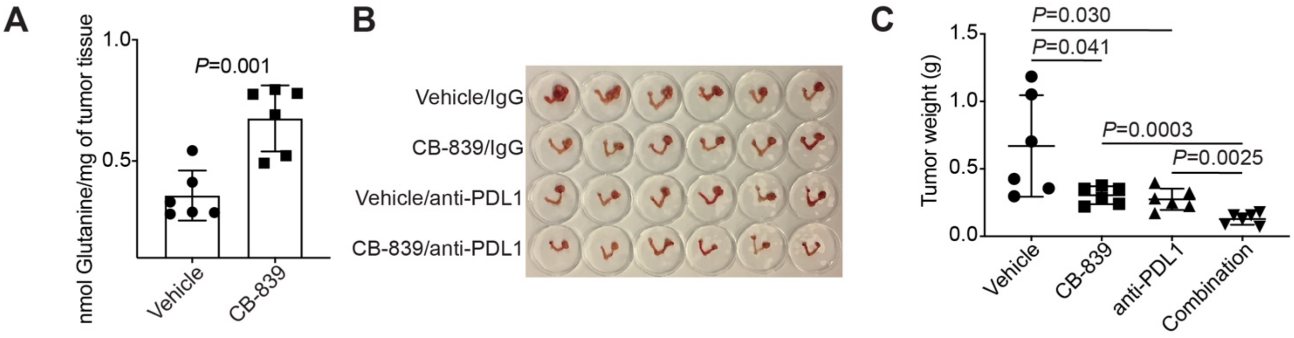

Fig. 10: Glutaminase inhibition in combination with immune checkpoint blockade suppresses the growth of *Arid1a/Pik3ca* OCCC.
A, Glutamine levels in the OCCCs developed from the *Arid1a/Pik3ca* genetic mouse model treated with vehicle control or CB-839 (n=6 mice/group). **B-C,** Mice bearing *Arid1a/Pik3ca* OCCCs were randomized into four indicated treatment groups. Shown are images of reproductive tracts with tumors from the indicated groups at the end of treatment (**B**). Tumor weight was measured as surrogate for tumor burden (**C**) (n=6 mice/group). Error bars represent mean with s.d. in **A** and **C**. *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?
In the next reporting period, we will determine whether the combination extends the survival of tumor bearing mice. In addition, we will correlate the observed changes in tumor burden with changes in immune cells.

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
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. 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 senescent cells and promotes age-associated inflammation. *Nature Aging*, under review
2. Lombardi S, Goldman AR, Tang HY, Kossenkov AV, Liu H, Zhou W, Herlyn M, Lin J, Zhang R (2023). Targeting fatty acid reprogramming suppresses CARM1-expressing ovarian cancer. Cancer Research Communications, 3(6): 1067-1077. PMCID: PMC10281290.
3. 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 (2023). Targeting the mevalonate pathway suppresses ARID1A-inactivated cancers by driving pyroptosis. *Cancer Cell*, 41(4): 740-756. PMCID: PMC10085864.
4. Liu H, Lin J, Zhou W, Moses R, Dai Z, Kossenkov A, Drapkin R, Bitler BG, Karakshev S, Zhang R (2022). KDM5A inhibits anti-tumor immune response through downregulation of antigen presentation pathway in ovarian cancer. *Cancer Immunology Research*, 10(8): 1028-1038. PMCID: PMC9357105
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7. 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.
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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
Invention Report Number: 9340401-23-0005
Title: INHIBITION OF KDM5A FOR PROMOTING ANTIGEN PRESENTATION, INCREASING CD8+ T CELL INFILTRATION AND BOOSTING ANTI-TUMOR IMMUNE RESPONSE
Date submitted in iEdison: 6/15/2023

Other Products
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

09/30/22-09/29/23

Name:	Qin Liu
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	.36 cm
Contribution to Project:	Supervised the study.
Funding Support:	This award

09/30/22-01/31/23

Name:	Rugang Zhang
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-7255-2360
Nearest person month worked:	.2 cm
Contribution to Project:	Supervised the study.
Funding Support:	This award

09/30/22-01/31/23

Name:	Shruthi Sriramkumar
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2.5 cm
Contribution to Project:	Performed the study.
Funding Support:	This award

09/30/22-01/31/23

Name:	Federica Severi
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	.6 cm

Contribution to Project:	Performed the study.
Funding Support:	This award

02/01/23-09/29/23

Name:	Rugang Zhang
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-7255-2360
Nearest person month worked:	.24 CM
Contribution to Project:	Supervised the study
Funding Support:	This award

02/01/23-09/29/23

Name:	Hao Nie
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6.0
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. Qin Liu:

End participation in “Integration of Biomarker Signatures from Peripheral Blood for Diagnosis, Prognosis, Remission and Recurrence of Lung Cancer”

Termination of “Rational approaches to melanoma therapy”

Termination of “Role of an Integrator-EGR axis in the regulation of myeloid enhancers”

Ended participation in “Synthetic lethality-based combination approaches to ARID1A mutation in ovarian cancer”

Ended Participation in “Define Novel Host Biomarkers and Mechanisms of Post-treatment Control of HIV”

Ended participation in “Mechanistic basis and therapeutic strategies for ARID1A mutation in ovarian cancer”

Ended participation in “Metabolic basis of ARID1A-mutated ovarian cancer”
Termination of “A First-in-Human Phase I Clinical Trial of Mitochondrial-Targeted Hsp90 Inhibitor”

Ended participation in “Therapeutic Targeting Mevalonate Pathway in ARID1A-Mutated Ovarian Cancer”

Activation of “Dissecting Phenotype Switching in Early Stage Melanomas”

Activation of “Glycomic Modulation of Gut Microbiome During HIV Infection”

Activation of “The genetics of tumor suppression by p53”

Activation of “Targeting the Epigenetic and Metabolic Control of EBV-Epithelial Cancers”

Activation of “Rational approaches to melanoma therapy”

Dr. Rugang Zhang has accepted a position in The University of Texas MD Anderson Cancer Center in Feb. 2023 and Dr. Qin Liu has since became the PI of the award.

Dr. Rugang Zhang:

Activation of “Epigenetic approaches to epithelial ovarian cancer” effective 01/2023

End participation in “Consolidated Basic Cancer Research Program” effective 02/2023

Termination of “JHU Spore in Ovarian Cancer” effective 07/2023

Termination of “Phase I/Ib trial evaluating the safety and efficacy of BET inhibitor, ZEN003694 with PD-1 inhibitor, nivolumab with or without CTLA-A inhibitor, ipilimumab in platinum resistant ovarian cancer’ effective 02/2023.

Activation of “Signaling basis of senescence-associated secretory phenotype and its implications in epithelial ovarian cancer” effective 07/2023

Activation of “MDACC Ovarian SPORE – Project 4: Targeting p300 to overcome PARP inhibitor resistance induced by acidic tumor microenvironment effective 07/2023

What other organizations were involved as partners?
The University of Texas MD Anderson Cancer Center

8. SPECIAL REPORTING REQUIREMENTS

Award Chart

9. APPENDICES:

References

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2. Wu, S., et al., *ARID1A spatially partitions interphase chromosomes.* Sci Adv, 2019. **5**(5): p. eaaw5294.
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4. Eads, J.R., et al., *Phase I clinical trial of the glutaminase inhibitor CB-839 plus capecitabine in patients with advanced solid tumors.* Journal of Clinical Oncology, 2018. **36**(15).
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11. Nabe, S., et al., *Reinforce the antitumor activity of CD8(+) T cells via glutamine restriction.* Cancer Sci, 2018. **109**(12): p. 3737-3750.

OC190181 : Metabolic approaches for ARID1A-mutated ovarian cancer

PI: Qin Liu, Wistar, PA **Budget:** $803,700.00

Topic Area: Ovarian Cancer Research Program (OCRP) Investigator-Initiated Research Award

Mechanism: W81XWH-19-OCRP-IIRA

Research Area(s): *1st 0105 - Tumor Suppressor Genes, 2nd 0805 - Targeted Therapies
Award Status: 09/30/2020 – 09/29/2024 (1yr NCE)

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. Emerging evidence supports the idea that the SWI/SNF complexes play a critical role in the tumor metabolism. Our preliminary data show that ARID1A inactivation decreases glucose uptake and creates a dependence on the glutamine metabolism. Indeed, ARID1A inactivation sensitizes ovarian cancer to glutaminase (GLS) inhibition using a clinically applicable GLS inhibitor such as CB-839. In addition, there is evidence to support that the SWI/SNF complex inactivation renders tumors susceptible to checkpoint inhibition. Our preliminary data show that ARID1A mutation sensitizes ovarian cancer to anti-PD-L1 treatment. This is consistent with a recent publication showing that ARID1A mutation increases the PD-L1 expression in cancer cells. Notably, the competition for glutamine in the tumor microenvironment can suppress T cell proliferation. Consistently, the inhibition of GLS activity in tumor cells potentiate T cell proliferation without suppressing T cell function. Together, these findings raised the possibility that the inhibition of GLS activity in ARID1A-mutated ovarian cancer will not only suppresses the growth of ARID1A mutant cancer cells but also potentiate T cell proliferation and thus enhance checkpoint blockade therapy. Our **central hypothesis** is that ARID1A-mutated ovarian cancer can be therapeutically eradicated by the combination of a clinically applicable glutaminase inhibitor such as CB-839 and anti-PD-L1 immune checkpoint blockade.

Specific Aims:

Specific Aim 1: To investigate the mechanism underlying the dependence of ARID1A mutation on glutamine metabolism.
Specific Aim 2: To develop a novel therapeutic approach for ARID1A-mutated ovarian cancer by combining a clinically applicable glutaminase inhibitor and anti-PD-L1.

OC190181 : Metabolic approaches for ARID1A-mutated ovarian cancer

PI: Qin Liu, Wistar, PA **Budget:** $803,700.00

Topic Area: Ovarian Cancer Research Program (OCRP) Investigator-Initiated Research Award

Mechanism: W81XWH-19-OCRP-IIRA

Key Accomplishments and Outcomes:

Publications:

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7. 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.
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9. 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). Therapeutic targeting glutamine dependence in SWI/SNF-inactivated cancers. Nature Cancer, 2: 189-200. PMCID: PMC8168620.

Patents:

Invention Report Number: 9340401-23-0005
Title: INHIBITION OF KDM5A FOR PROMOTING ANTIGEN PRESENTATION, INCREASING CD8+ T CELL INFILTRATION AND BOOSTING ANTI-TUMOR IMMUNE RESPONSE
Date submitted in iEdison: 6/15/2023

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