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PRINCIPAL INVESTIGATOR: Jeremy Chien, Ph.D.

CONTRACTING ORGANIZATION: University of California, Davis, Sacramento, CA

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Jeremy Chien

E-Mail: jrchien@ucdavis.edu

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University of California, Davis
Department of Biochemistry and Molecular Medicine
4635 2nd Ave, Room 2006
Sacramento, CA 95817

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14. ABSTRACT

This report consists of work-in-progress research results to target ovarian cancer by inducing proteotoxicity through inhibition of VCP by a small molecule inhibitor CB-5083. We report results of dose-finding studies and pharmacodymic markers associated with CB-5083 treatment. We also show that this compound is synergistic with HDAC6 inhibitor ACY-241 and report a pharmacodynamic marker associated with ACY-241.

15. SUBJECT TERMS

ER stress, proteotoxicity, protein homeostasis, HDAC6 inhibitor, ACY-241, ovarian cancer, patient-derived organoids

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1. INTRODUCTION

Acquired resistance to platinum-based chemotherapy is a major clinical issue in the treatment of ovarian cancer, and new strategies to overcome this obstacle are needed to improve overall survivorship of patients with advanced-stage ovarian cancer. Recent advances in PARP inhibitors (**PARPi**) as maintenance therapy for ovarian cancer are improving the overall survivorship. Unfortunately, recurrences from these treatments are still common, and a five-year survival rate is unacceptably low. **One of the main goals of my research program is to develop new therapies that enhance the efficacy of platinum and PARPi-based therapies and prolong the clinical response produced by these drugs**. Toward this goal, we are developing novel experimental therapeutics that target protein quality control (**PQC**) pathways in ovarian cancer. The rationales for targeting this pathway in cancer are many: (i) prior studies indicate PQC pathways are critical for genetic diversity and adaptability due to the fact that they buffer the potentially detrimental effects of genetic variations until such effects are favorable due to changing selection pressure [1, 2]; (ii) thus by an extension of this principle, the buffering mechanisms of PQC allow genetic variations to exist in the population that can then provide adaptive responses to drug treatment, leading to drug resistance and survival of cancer cells; (iii) finally, recent functional genomic studies indicate the disruption of genes involved in PQC produce synthetic lethal effects in cancer cells due to the fact that cancer cells are more dependent on PQC pathways for improving their fitness under a highly deranged genomic landscape [3]. In particular, a recent report indicates that the disruption of the valosin-containing protein (**VCP**) gene that encodes for a protein involved in the PQC pathway resulted in lineage-specific vulnerability in ovarian cancer cells compared to other cancer types [4].

Based on the evidence from these studies, we proposed that drugs that inhibit VCP activity are effective against ovarian cancer. VCP is an enzyme that uses its ATPase activity to produce mechanical force to separate unfolded protein aggregates or proteins that are tagged for destruction from the protein complexes and facilitate their destruction through the proteasome system [5]. VCP inhibitors bind to the ATP binding pocket within VCP protein and inhibit the ATPase activity thereby inhibiting the PQC function of VCP [6-9]. Additionally, recent studies indicate that VCP plays a critical role in DNA damage and repair functions. In particular, VCP extracts polyubiquitinylated PARP1 trapped by PARP inhibitors, and thus VCP inhibitors act synergistically with PARP-trapping inhibitors in suppressing the growth of ovarian cancer cells [10].

In the past few years, we reported our initial studies in which we observed several VCP inhibitors are effective against ovarian cancer cell lines (**Bastola et al, Mol Oncol, 2016, PMID:27729194**) [11]. We provided a proof of concept that the PQC pathway can be targeted in ovarian cancer. We also reported additional follow-up studies in three manuscripts (**Bastola et al, Cell Death & Discovery, 2017, PMID:29367883; Bastola et al, Cell Death & Disease, 2018, PMID: 29348605; and Bastola et al, AAPS Journal, 2018, PMID: 3051644**) [12-14]. Studies investigating the potential synergistic drug-drug interaction between VCP inhibitors and atypical ER stress-inducer mifepristone were included in previous annual report, and we are working toward submitting a manuscript reporting these results for a peer review and publication. Also in previous reports, we included the resulting of ongoing studies to investigate the molecular mechanisms that produce drug synergies between VCP inhibitors and platinum agents or PARP inhibitors as well as other ER stress inducers. In this interim report before the final report, we focus on preliminary in vivo dose-finding studies that will allow us to design the final in vivo studies

testing the efficacy of combining VCP inhibitor CB-5083 and HDAC6 inhibitor (an ER stress inducer) ACY-241.

2. KEYWORDS

Ovarian cancer; VCP inhibitors; Protein homeostasis; Proteotoxic stress; ER stress; HDAC6 inhibitor; ACY-241; Experimental therapeutics; DNA repair; PARP inhibitors

3. ACCOMPLISHMENTS

Major Goals of the Project

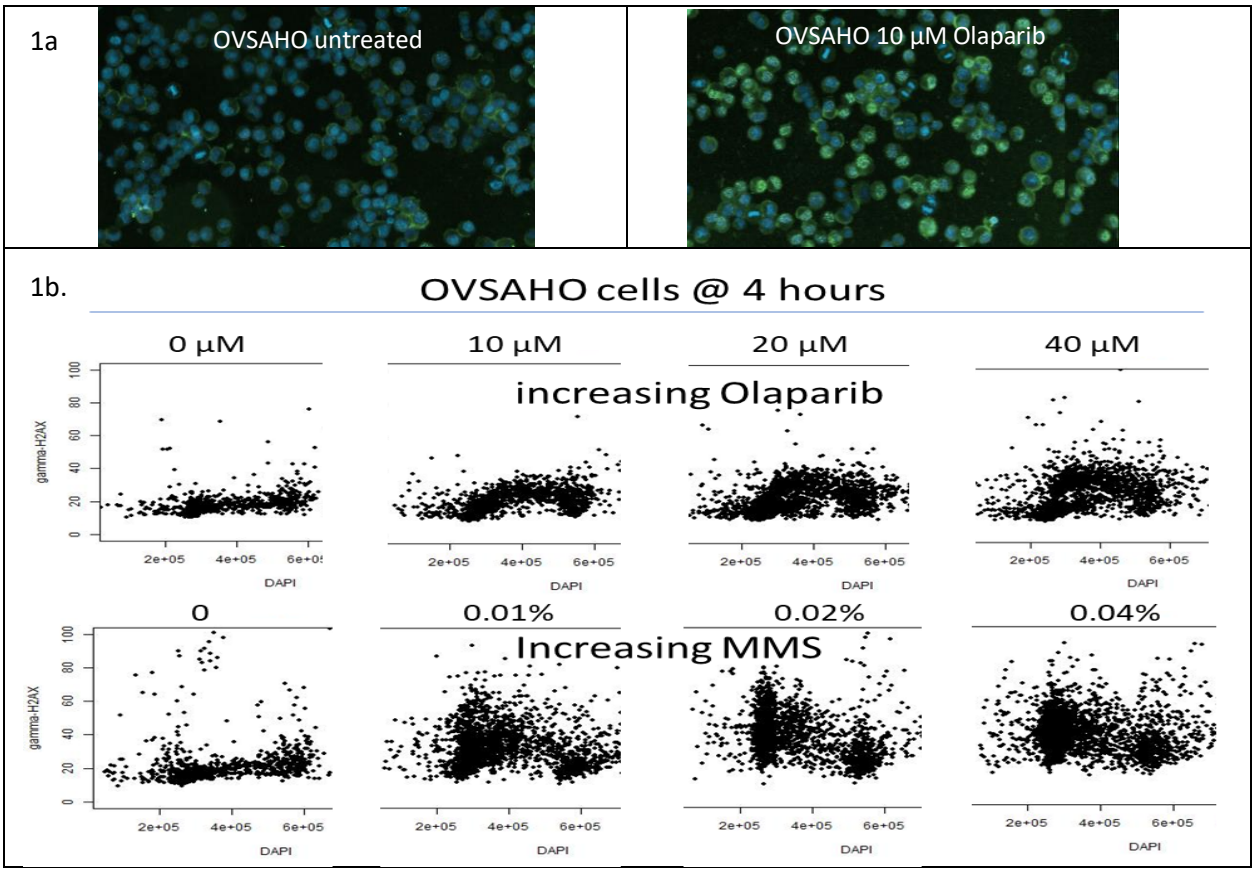
- **Specific Aim 1**: *Determine the effect of VCP inhibitors on cisplatin sensitivity.*
- **Specific Aim 2**: *Determine the combined effect of VCP inhibitors and other ER stress inducers in ovarian cancer.*

Accomplished tasks included in the last year report

I. Effect of VCP inhibitor on DNA damage signaling (related to Specific Aim 1): Functional deficiency in VCP compromises double-strand break repair and results in neuronal demise [15]. Furthermore, VCP ATPase activity is essential for the extraction of ubiquitinated substrates during the DNA repair process [16]. Therefore, VCP inhibition may cause deficiencies in DNA repair function and may sensitize ovarian cancer cells to cisplatin. To test this hypothesis, we previously treated several ovarian cancer cell lines with cisplatin and VCP inhibitors DBeQ and CB-5083. We then determined the combination index at various concentrations and showed that VCP inhibitors were synergistic with cisplatin in suppressing cell viability at specific drug combinations (data presented in previous reports).

To dissect the mechanisms contributing to the drug synergies, we determined the extent to which VCP inhibitor CB-5083 interferes with ubiquitination of nuclear proteins and enhances DNA damages assessed through phospho-H2AX staining. It should be noted that histone H2AX phosphorylation (phospho-H2AX) has been used as a marker for DNA damage because H2AX is phosphorylated at DNA lesions [17].

OVSAHO ovarian cancer cells were grown at 37°C, 5% CO_2 in RPMI-1640 with 10% FBS. $2.0x10^3$ cells/well were plated in clear-bottom plastic 96-well plates and grown overnight to approximately 50% confluence and treated with drug concentrations and combinations as indicated. Four hours after treatment, cells were fixed in 3% formaldehyde/PBS for 15 minutes, permeabilized and blocked with 0.2% Triton X-100 and 1.5% BSA in PBS, then incubated overnight in primary antibodies (BioLegend, γH2AX mouse monoclonal antibodies, 1/500) at 4°C in 0.2% Triton X-100/1% BSA PBS. After 3 washes in Tris-buffered saline with 0.1% Tween-20 (TBST), cells were incubated with anti-mouse secondary antibodies conjugated to Dylight 488 (1/750 dilution) for 1 hour at room temperature. After 3 TBST washes, cells were incubated in DAPI stain to 5 µg/mL in PBS. Images and image data were obtained using Molecular Dynamics ImageXpress Pico at 20X magnification with 2-8 images per well and typically 3 wells per drug condition. Individual cell fluorescence data tables were transformed using Perl scripts to map experiment conditions into formats compatible for R statistical analysis using R Studio. R plotting and analysis used readr, dplyr, forcats and ggplot2 libraries.



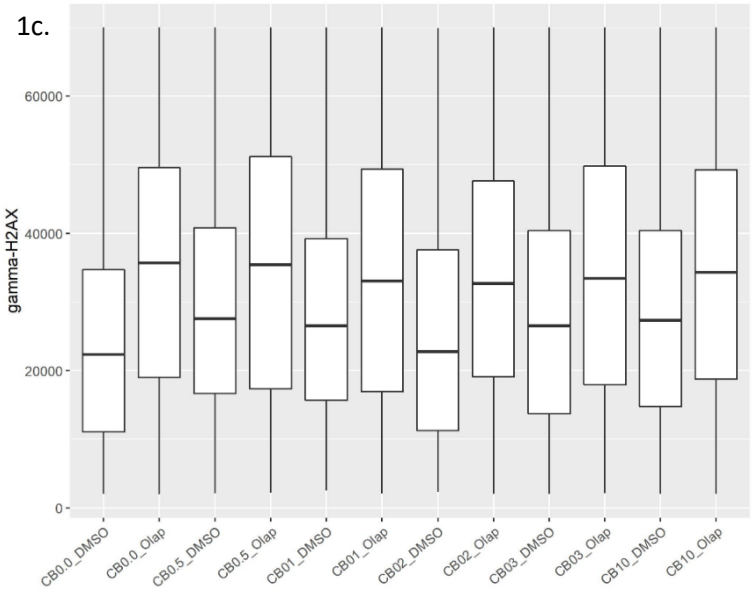


Figure 1. Single-cell quantification of γH2AX (phospho-H2AX) signal in response to Olaparib and Olaparib + CB-5083 treatment. (a) Representative OVSAHO cell imaging after 4 hours of treatment with DMSO vehicle versus 10 µM Olaparib (γH2AX primary antibodies and anti-mouse secondary antibodies conjugated to Dylight 488). (b) Scatter diagram of γH2AX signal (Y-axis) versus DAPI stain (X-axis) intensity (as a marker of DNA content and cell cycle phase). Methyl methanesulfonate (MMS) is used as a positive control to induce DNA damages. (c) OVSAHO nuclear γH2AX signal in response to increasing CB-5083 concentrations, with and without 10 µM Olaparib (Olap). CB0.0, CB0.5, CB01, CB02, CB03, and CB10 denote 0, 0.5, 1, 2, 3, and 10 µM CB-5083 respectively.

Interpretation of results: Considering that Olaparib inhibits PARP1-mediated repair of single-strand DNA damages, PARP inhibitor Olaparib is expected to increase DNA damages, and accordingly, we observed an increase in phospho-H2AX signal (Figure 1a, 1b, and 1c). We also observed an increase in phospho-H2AX signal (fluorescence intensity) following the treatment with various concentrations of CB-5083 (Figure 1c). Given that CB-5083 was not previously reported to induce DNA damages, this observation was novel and unexpected. However, considering that VCP is involved in DNA damage response, its inhibition by CB-5083 is likely to impair DNA damage response and consequently leave damage sites unrepaired, leading to increased DNA damages and phospho-H2AX staining. Given the role of VCP in DNA repair, we expected CB5083 and Olaparib combination would enhance DNA damages and increase phospho-H2AX immunofluorescence. Surprisingly, we did not see an increase in phospho-H2AX immunofluorescence. We suspect that both VCP and PARP1 are involved in DNA repair processes and therefore inhibition of both proteins may be redundant. However, it is also likely that VCP and PARP1 play a role in different stages of the repair process, and inhibition of both may alter the repair dynamics. Unfortunately, due to the limited time frame of the current study (4 hours after co-treatment), we could not assess the dynamics of DNA repair signaling.

II**. Effect of VCP inhibitor on K48 ubiquitin signal**: Given the role of VCP in the extraction of ubiquitinylated proteins from the complexes involved in DNA damage signaling [16, 18-20], the subsequent degradation through proteasome system, and the resolution of DNA damage signaling, we checked the extent to which K48 ubiquitin signals were affected by exposure to 4 hours of CB-5083 in the presence of select DNA repair inhibitors or DNA damaging agents. K48 ubiquitin nuclear immunofluorescence was quantified using the ImageXpress Pico (Figure 2).

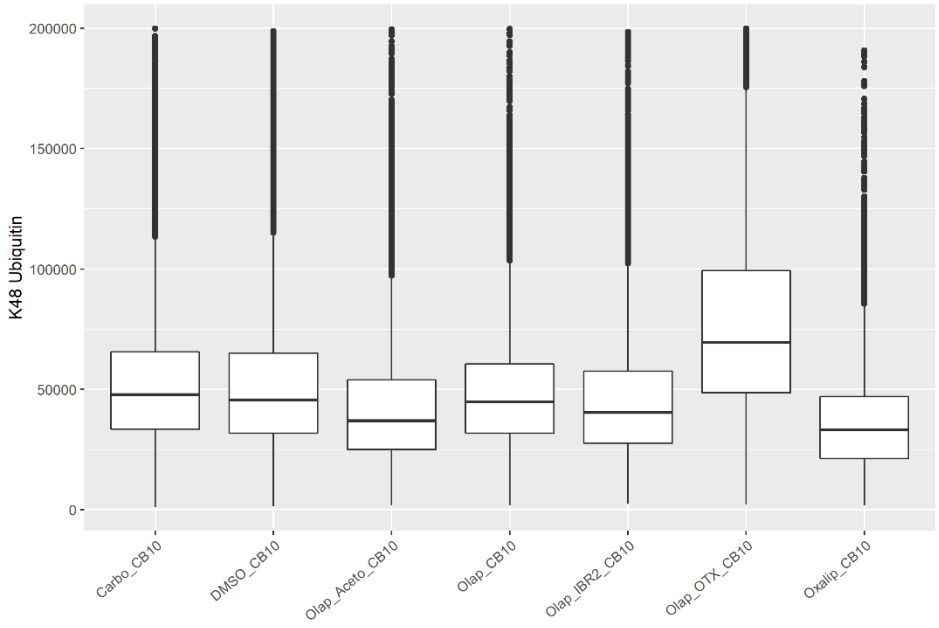


Figure 2. Single-cell quantification of K48 ubiquitin signal in response to DNA damage inducers and DNA repair inhibitors plus 10 μM CB-5083 (CB). Abbreviations: carboplatin (carbo); dimethyl sulfoxide (DMSO); Olaparib (Olap); diabetes drug and MUTYH degrader acetohexamide (Aceto) [21]; RAD51 inhibitor IBR2 [22]; APE1 antagonist O-tetrahydro-2H-pyran-2yl hydroxylamine (OTX) [23]; and oxaliplatin (Oxalip). At least 5000 cells/group were analyzed for K48 ubiquitin quantification.

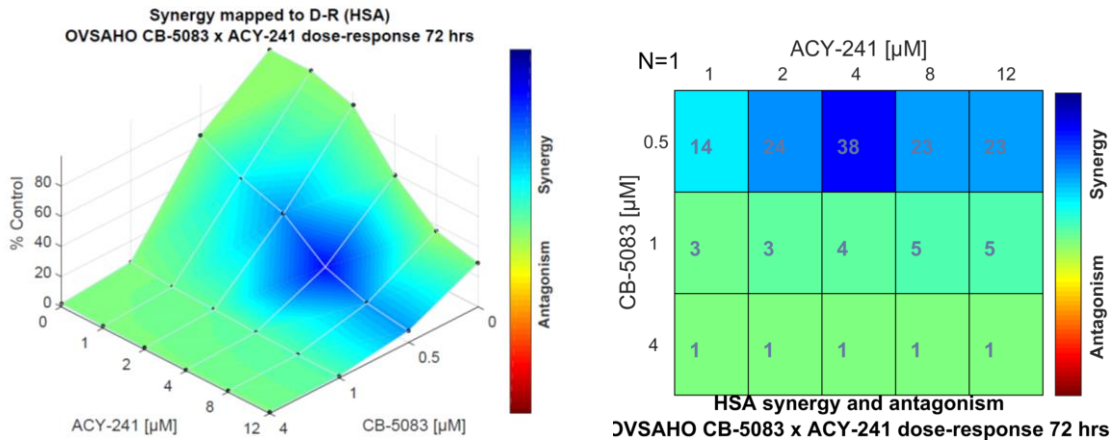
Interpretation of results: Ubiquitin and ubiquitin-like modification, including SUMO (small ubiquitin-like modifier) of specific proteins play an important role in DNA damage response by serving as a recruitment signal for DNA repair factors [18]. VCP recognizes ubiquitylate proteins and extract them from the damage sites to facilitate efficient exchange of repair factors and processing [16, 19, 20]. Therefore, CB-5083 is expected to increase ubiquitylation of proteins by inhibiting VCP, thereby decreasing DNA repair efficiency. Consistent with its expected effect, we observed an increase in K48 ubiquitylation signal in the nuclei of cells treated with OTX (Figure 2).

III. The combined effect of VCP inhibitors and ER stress inducers (related to Specific Aim 2): Given that VCP inhibitors induce ER stress and unfolded protein response (UPR), which when unresolved promotes cytotoxicity, we tested the extent to which VCP inhibitors are synergistic with other ER stress inducers. In previously published studies, we reported that VCP inhibitors show synergistic cytotoxic effects with salubrinal which acts as a modulator of the ER stress response. In the previous annual report, we included additional studies that demonstrate synergistic cytotoxic effects with other ER stress inducers, such as mifepristone and ISRIB. To advance better clinical applicability of these studies, we further tested the potential synergistic cytotoxic effects with an ER stress inducer that is in several Phase I/II clinical trials, namely ACY-241. ACY-241 is a Histone Deacetylase 6 (HDAC6) inhibitor that is involved in protein aggregate clearance. HDAC6 inhibition results in the accumulation of protein aggregates, ER stress, and proteotoxicity. Therefore, we tested its potential synergy with CB-5083.

OVSAHO, OVCAR3, and TOV21G ovarian cancer cells were grown at 37°C 5% CO_2 RPMI 1640 with 10% FBS. Cells were plated at 5000 cells per well in 96 well plates and grown overnight, then treated with the indicated concentrations of drugs, with 4 replicate wells per condition. After 48 or 72 hours cell viability was measured using CellTiter-Glo reagent (Promega) with a luminescent plate reader. Assessment of drug combination synergy based on the average value of 4 wells is shown using Combenefit software [24] with the HSA synergy algorithm [25].

Dose-response for CB-5083 treatment alone yielded an EC_{50} of 0.57, 1.40, and 0.65 µM for OVSAHO, OVCAR3, and TOV21G cell lines, respectively. ACY-241 treatment alone resulted in an EC_{50} of 4.85, 2.54, and approximately 38 µM for OVSAHO, OVCAR3, and TOV21G cell lines, respectively. Combenefit graphical representation of antagonism/synergy is shown in Figure 3. At CB-5083 concentrations of 0.5-1 µM, ACY-241 has a synergistic effect on cell viability at approximately 2-4 µM, as measured 48-72 hours after drug treatment.

A



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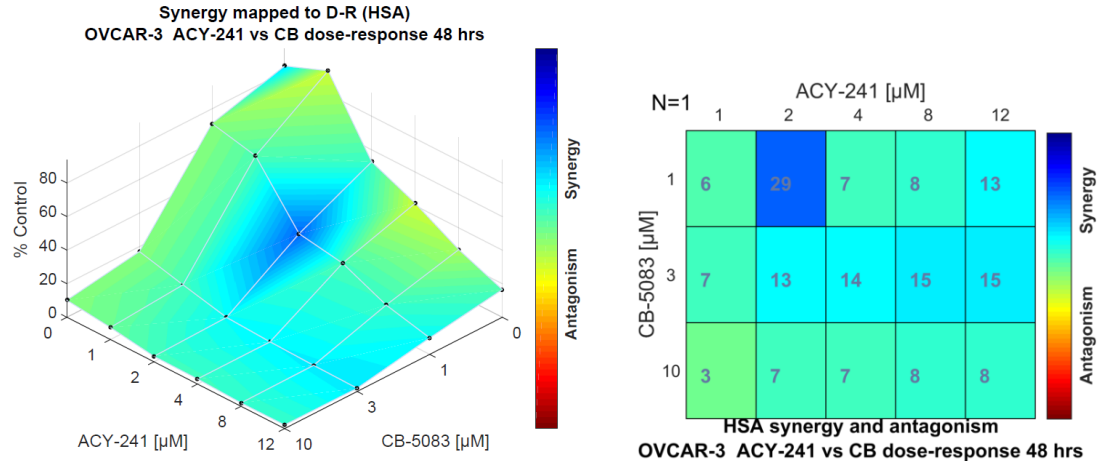

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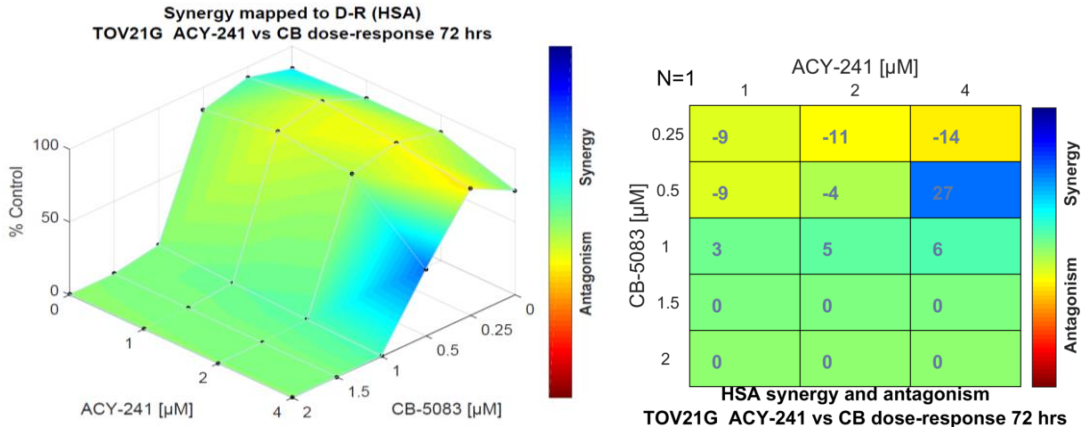

Figure 3. A-C. Dose-response and synergy for CB-5083 and ACY-241 in three ovarian cancer cell lines. Synergy scores above 10 are considered synergistic.

Accomplished tasks in this report

In vivo dose-finding studies (related to Specific Aim 2): In the past few months, we collaborated with Leidos, Inc., investigators who have developed genetically engineered mouse model (GEMM) of ovarian cancer to perform in vivo dose-finding studies. Dr. Zoe Ohler group from the Leidos Inc., and the National Cancer Institute have developed a GEMM model by disrupting p53 (p53 null) and Rb (suppressed) with or without BRCA1 or BRCA2 disruption. These mice (TgK18GT121;p53$^{fl/fl}$) developed serous epithelial ovarian cancer that closely resembles the human disease. Cell lines established from the tumors of these mice can be used to produce tumor allograft for in vivo drug testing. Considering that these allograft tumor models have intact immune system, these models are useful in testing the potential immune modulatory effects of VCP inhibitors and ER stress inducers. Considering that both CB-5081 and ACY-241 induce ER stress and that ER stress inducers can promote immunogenic cell death, we plan to explore potentially beneficial immune-stimulatory effects of these agents in treating tumor allografts produced from the GEMM.

Results: In the Tolerability and dose-finding studies, Dr. Ohler and her group have treated the mice with CB-5083 alone (per oral), ACY-241 alone (per i.p.), or 2-drug combination. After the 3rd or 4th doses of these drugs, mice were euthanized, and several tissues were collected to determine on-target pharmacodynamic of these agents. ACY-241 is a histone deacetylase inhibitor, and its on-target inhibition of HDAC6 results in the increased acetylation of tubulin. CB-5083 is a VCP inhibitor, and its on-target inhibition of VCP results in the increased expression of ATF4 and ubiquitinylation of cellular proteins because VCP participates in the extraction of ubiquitinylated proteins and degradation of these proteins through proteasome system. As expected, these targets are affected in several tissues collected from mice with tumor allografts (Figure 4).

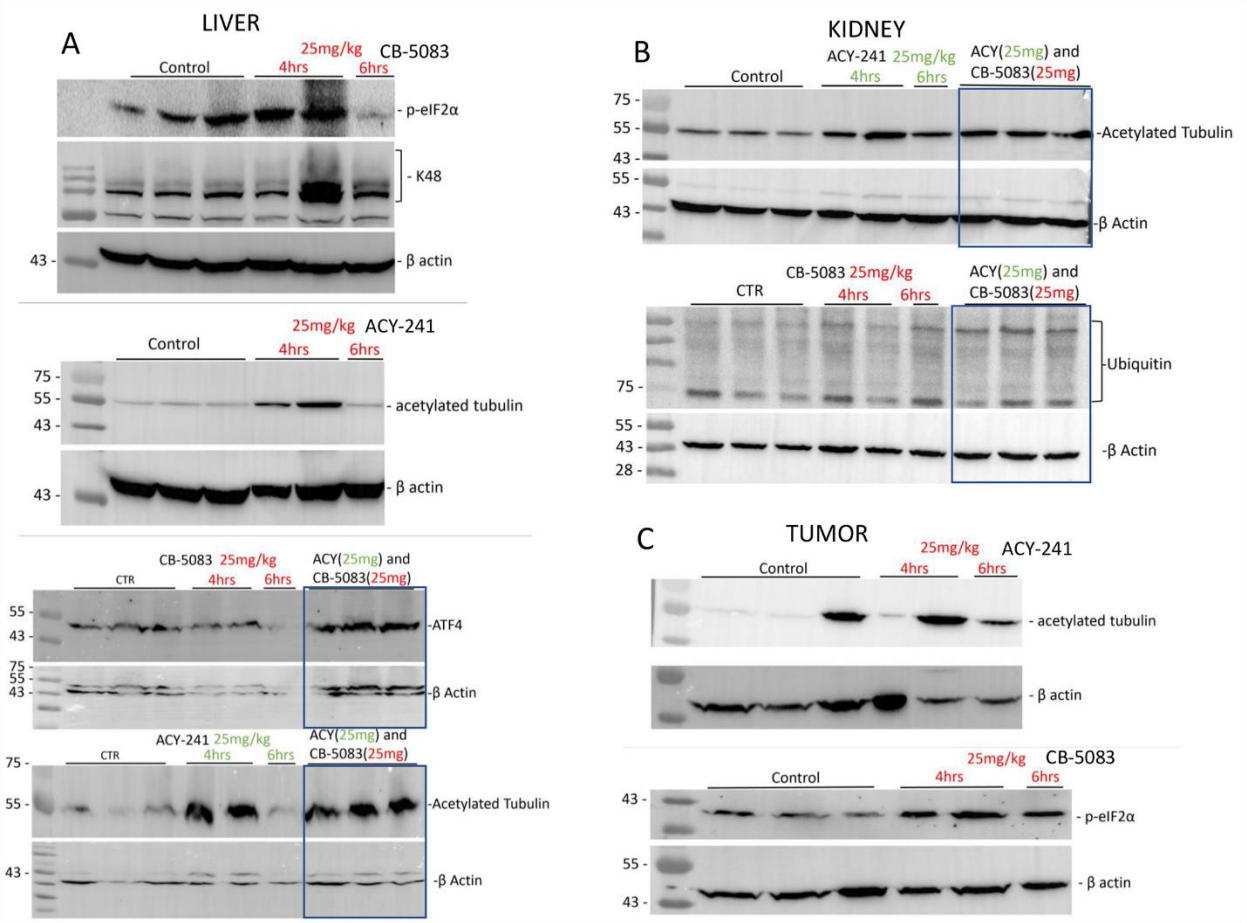

Figure 4. A. CB-5083 treatment results in the increased phosphorylation of eIF2a and ubiquitination of proteins in liver tissue samples. ACY-241 treatment results in the enhanced acetylation of tubulin (a known target of HDAC6) in liver tissue samples. The combination treatment increases the expression of ATF4 (a pharmacodynamic marker of CB-5083) and acetylated tubulin (a pharmacodynamic marker of ACY-241). B-C. Similar results are observed in kidney tissue samples and tumor samples.

Follow-up studies: Based on these dose-finding studies, we selected 25 mg/kg for 2-drug combination studies. We plan to complete the treatment response studies in the next few months, and the results of these studies will be included in the final report.

CONCLUSION: Here, we showed the potential therapeutic benefit of targeting the PQC pathway in ovarian cancer by inhibiting VCP. VCP inhibitors, such as CB-5083, produce unresolved unfolded protein response that eventually initiates caspases-mediated cell death [11]. Furthermore, we showed that VCP inhibitors can be combined with salubrinal potentiating the effect of VCP inhibitors [11]. Our previous report also included the results of a 2-drug combination targeting HDAC6 with ACY-241 and VCP with CB-5083, known inducers of unfolded protein response and ER stress. In current phase, we are focusing on obtaining confirmatory in vivo data for synergistic drug interactions between ACY-241 and CB-5083.

Future studies will focus on understanding the molecular mechanisms contributing to drug synergies between ACY-241 and CB-5083. In addition, recent studies indicate that VCP play a critical role in the removal of PARP1 trapped by PARP inhibitors and that VCP inhibition enhances the cytotoxic effect of PARP inhibitors [10]. Therefore, future studies will also focus on potential drug synergies between VCP inhibitor CB-5083 and PARP inhibitors.

KEY RESEARCH ACCOMPLISHMENTS:

- Collaborators at Leidos, Inc., have completed the dose-finding studies testing the combination of CB-5083 and ACY-241 in a GEMM ovarian cancer.
- We received tissue specimens from our collaborators at Leidos, and we confirmed cellular targets were affected by the dose, route, and administration of two drugs.

Completion status in reference to revised SOW

Specific Aim 1: Determine the effect of VCP inhibitors on cisplatin sensitivity	Completion status	Site 1
Major Task 1		
Subtask 1: *Determine the effect of CB-5083 on the localization of BRCA1 and P53BP1 following DNA damage* Cell lines: same as in Subtask1	80%	UCD
Subtask 2: Obtain IACUC and subsequent ACURO approval (We are currently processing contractual agreement.)	100%	CAPR Leidos
Subtask 3: *In vitro effects of CB-5083 and ACY-241 in ovarian cancer cell lines*	100%	UCD
Subtask 4. In vivo effect of CB-5083 and ACY-241 in syngeneic ovarian cancer mouse model.	80%	CAPR Leidos
Subtask 5: Immunohistochemical and molecular biology studies of tissue samples collected from Subtask 4 for therapeutic engagement.	50%* (See Figure 4)	UCD

*****We completed histology and immunoblot analysis of tissues collected from initial dose-finding studies that demonstrated targets were engaged by the dose we selected. We are awaiting additional tissue samples from Subtask4 for the treatment response studies.

REPORTABLE OUTCOMES AND DISSEMINATION OF RESULTS:

Published manuscripts

- None

Manuscripts (under review)

- Bastola et al, Multiple components of protein homeostasis pathway can be targeted to produce drug synergies with VCP inhibitors in ovarian cancer. Pharmacological Research (submitted in April 2022).

Published abstracts

- None

Oral Presentation:

- Undergraduate student researcher Sydney Woods presented her research studies at the Undergraduate Research Symposium

Opportunities for trainees:

- During this reporting period, three undergraduate researchers participated in the research focusing on advancing treatment options for patients with ovarian cancer.

4. IMPACT

Our observation that CB-5083 and ACY-241 show synergistic drug interactions is likely to impact future development of this combination of drugs to treat ovarian cancer. However, before this combination is advanced to treatment of ovarian cancer, it is important to validate that synergistic drug activities are observed under in vivo models. Therefore, the next step in mouse studies is critical to fully evaluate the potential impact of our in vitro observation. We are currently performing these in vivo studies.

There is nothing to report for the impact on other disciplines.

There is nothing to report for the impact on technology transfer.

There is nothing to report for the impact on society beyond science and technology?

5. CHANGES/PROBLEMS

Progress was delayed due to restrictions on research activities imposed by the COVID-19 pandemic response at both institutions (UC Davis and Leidos, Inc). These delays are now resolved, and in vivo treatment studies are underway.

6. PRODUCTS

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Jeremy Chien
Project Role:	Principal Investigator
ORCID ID:	0000-0003-4744-8374
Nearest person month worked:	2
Contribution to Project:	Dr. Chien supervised all aspect of research studies, analysis of data, and preparation of manuscripts and reports.
Funding Support:	

Name:	Alan Raetz
Project Role:	Postdoctoral Fellow
ORCID ID:	
Nearest person month worked:	4
Contribution to Project:	Dr. Raetz performed cytotoxicity assays and immunofluorescence studies.
Funding Support:	

Name:	Caili Tong
Project Role:	Research Associate
ORCID ID:	
Nearest person month worked:	2
Contribution to Project:	Ms. Tong performed cell culture, gene expression, and molecular biology studies.
Funding Support:	

Change in the active other support of the PI/PD:

Nothing to report.

Other organizations involved as partners:

We are collaborating with Dr. Zoe Ohler from the Leidos Biomedical and the National Cancer Institue to assist us with mouse studies to determine synergistic drug interactions between CB-5083 and ACY-241.

Organization Name: Leidos Biomedical

Location: 1050 Boyles Street, Frederick, MD 21702

Partner's contribution to the project: Dr. Ohler is performing in vivo drug studies utilizing a mouse model of ovarian cancer that she helps developed and maintained for anti-cancer drug testing. We will perform follow up studies using tissue specimens collected from these treated mice to determine molecular mechanisms elicited by these drugs.

Other: This Cooperative Research and Development Agreement (CRADA) is supported by the grant.

References

1. Queitsch, C., T.A. Sangster, and S. Lindquist, *Hsp90 as a capacitor of phenotypic variation.* Nature, 2002. **417**(6889): p. 618-24.
2. Rutherford, S.L. and S. Lindquist, *Hsp90 as a capacitor for morphological evolution.* Nature, 1998. **396**(6709): p. 336-42.
3. Marcotte, R., et al., *Essential gene profiles in breast, pancreatic, and ovarian cancer cells.* Cancer Discov, 2012. **2**(2): p. 172-89.
4. Cheung, H.W., et al., *Systematic investigation of genetic vulnerabilities across cancer cell lines reveals lineage-specific dependencies in ovarian cancer.* Proc Natl Acad Sci U S A, 2011. **108**(30): p. 12372-7.
5. Meyer, H. and C.C. Weihl, *The VCP/p97 system at a glance: connecting cellular function to disease pathogenesis.* J Cell Sci, 2014. **127**(Pt 18): p. 3877-83.
6. Chou, T.F., et al., *Reversible inhibitor of p97, DBeQ, impairs both ubiquitin-dependent and autophagic protein clearance pathways.* Proc Natl Acad Sci U S A, 2011. **108**(12): p. 4834-9.
7. Chou, T.F., et al., *Specific inhibition of p97/VCP ATPase and kinetic analysis demonstrate interaction between D1 and D2 ATPase domains.* J Mol Biol, 2014. **426**(15): p. 2886-99.
8. Chou, T.F., et al., *Structure-activity relationship study reveals ML240 and ML241 as potent and selective inhibitors of p97 ATPase.* ChemMedChem, 2013. **8**(2): p. 297-312.
9. Chou, T.F., et al., *Selective, reversible inhibitors of the AAA ATPase p97*, in *Probe Reports from the NIH Molecular Libraries Program*. 2010: Bethesda (MD).
10. Krastev, D.B., et al., *The ubiquitin-dependent ATPase p97 removes cytotoxic trapped PARP1 from chromatin.* Nat Cell Biol, 2022. **24**(1): p. 62-73.
11. Bastola, P., et al., *VCP inhibitors induce endoplasmic reticulum stress, cause cell cycle arrest, trigger caspase-mediated cell death and synergistically kill ovarian cancer cells in combination with Salubrinal.* Mol Oncol, 2016.
12. Bastola, P., et al., *Emerging Cancer Therapeutic Targets in Protein Homeostasis.* AAPS J, 2018. **20**(6): p. 94.
13. Bastola, P., et al., *Specific mutations in the D1-D2 linker region of VCP/p97 enhance ATPase activity and confer resistance to VCP inhibitors.* Cell Death Discov, 2017. **3**: p. 17065.
14. Bastola, P. and J. Chien, *Co-selected mutations in VCP: a novel mechanism of resistance to VCP inhibitors.* Cell Death Dis, 2018. **9**(2): p. 35.
15. Fujita, K., et al., *A functional deficiency of TERA/VCP/p97 contributes to impaired DNA repair in multiple polyglutamine diseases.* Nat Commun, 2013. **4**: p. 1816.
16. Torrecilla, I., J. Oehler, and K. Ramadan, *The role of ubiquitin-dependent segregase p97 (VCP or Cdc48) in chromatin dynamics after DNA double strand breaks.* Philos Trans R Soc Lond B Biol Sci, 2017. **372**(1731).
17. Sharma, A., K. Singh, and A. Almasan, *Histone H2AX phosphorylation: a marker for DNA damage.* Methods Mol Biol, 2012. **920**: p. 613-26.
18. Schwertman, P., S. Bekker-Jensen, and N. Mailand, *Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers.* Nat Rev Mol Cell Biol, 2016. **17**(6): p. 379-94.
19. Acs, K., et al., *The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks.* Nat Struct Mol Biol, 2011. **18**(12): p. 1345-50.
20. Meerang, M., et al., *The ubiquitin-selective segregase VCP/p97 orchestrates the response to DNA double-strand breaks.* Nat Cell Biol, 2011. **13**(11): p. 1376-82.

21. Mazouzi, A., et al., *Repair of UV-Induced DNA Damage Independent of Nucleotide Excision Repair Is Masked by MUTYH.* Mol Cell, 2017. **68**(4): p. 797-807 e7.
22. Zhu, J., et al., *A novel small molecule RAD51 inactivator overcomes imatinib-resistance in chronic myeloid leukaemia.* EMBO Mol Med, 2013. **5**(3): p. 353-65.
23. Luke, A.M., et al., *Accumulation of true single strand breaks and AP sites in base excision repair deficient cells.* Mutat Res, 2010. **694**(1-2): p. 65-71.
24. Di Veroli, G.Y., et al., *Combenefit: an interactive platform for the analysis and visualization of drug combinations.* Bioinformatics, 2016. **32**(18): p. 2866-8.
25. Tan, X., et al., *Systematic identification of synergistic drug pairs targeting HIV.* Nat Biotechnol, 2012. **30**(11): p. 1125-30.