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TITLE: A New Paradigm for the Treatment of Ovarian Cancer: The Use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy

PRINCIPAL INVESTIGATOR: Stephen B. Baylin, M.D.

CONTRACTING ORGANIZATION: The Johns Hopkins University Baltimore, MD 21287

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6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: Our overall goal remains to bring epigenetic therapy to have major impact for the management of advanced ovarian cancer (OC). This past year, we continue to make exciting advances in our pre-clinical work and interim results from our leveraged clinical trial is pending now for low dose therapy targeting DNA demethylation paired with immune checkpoint therapy. Our two relevant studies of mouse models are now published, a study of a serous ovarian cancer in which we have identified that the demethylating agent, 5-aza-cytidine (AZA) potently stimulates tumor immune attraction of T-cells to the tumor microenvironment (**PNAS, 2017**). The treatment paradigm involves a newly regimen we first derived for addition of a histone deactylase inhibitor (HDACi) in a study of mouse models for lung cancer (**Cell, 2017**). Further, we have just published work showing how an inhibitor of G9A, an enzyme mediating transcriptional repression, can augment the above AZA effects to induce the immune attraction parameters in OC cells (**Cancer Research, 2018**). All of the above findings continue to document how epigenetic therapy can potentially improve immune checkpoint therapy for OC.

15. SUBJECT TERMS – key words or phrases identifying major concepts Epigenetic therapy, advanced ovarian cancer, DNA demethylation, histone deactylases, immune checkpoint therapy, viral defense, immune cell attraction

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1. INTRODUCTION

We continue to pursue our goal to develop eventual "epigenetic" therapy strategies, with relatively low toxicities, which can potentially robustly extend the life expectancy of women with advanced ovarian cancer (OC). In this past year we have published a series of studies which continue to suggest how epigenetic therapy can reverse tumor immune evasion states and thereby potentially enhance the efficacy of immune checkpoint therapy. For the last year of our award, we will continue to concentrate on **Specific Aim 3:** *to study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance*. Our funded research leaders including Cindy Zahnow, Dennis Slamon, Drew Pardoll, and Peter Jones have contributed to our progress for **Major Task 1**: *to develop the in-vitro pre-clinical systems to outline the sensitivities and derive molecular signatures that track with these*, and **Major Task 2:** *to develop in-vivo pre-clinical systems to outline the potential efficacy of epigenetic therapy sensitization to immunotherapy for targeting checkpoints which drive immune tolerance*. *This progress was, and continues to be the impetus for having derived our ongoing leveraged clinical trial, headed by Dennis Slamon, which has now enrolled enough patients with* advanced *serous OC* to allow a pending interim analysis. The results will determine whether a phase 2 portion of the trial will move forward to determine the above outlined potential for epigenetic therapy to improve the efficacy of immune checkpoint therapy in OC. Samples from the trial will also be available during this last year for performance of a key goal of our funding, to perform correlative science biomarker studies to help us learn why we have or have not achieved any promising results and how to predict patient responses and personalize the therapy if warranted. We have also published our progress in **Specific Aim 4:** *Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC and:* **Major Task 1:** *Follow biochemical hypotheses for designing combinations of the epigenetic drugs used in all studies above with new agents targeting additional steps in chromatin control of gene expression.*

2. KEYWORDS

1) epigenetic therapy; 2) DNA demethylation; 3) histone deacetylases; 4) immune evasion; 5) immune checkpoint therapy; 6) immune attraction.

3. ACCOMPLISHMENTS

What were the major goals and objectives of the project?

The overall goals remain identical to those outlined in the original proposal and last year's progress report. We will particularly outline what has transpired during the past year in terms of where have focused our work and how we will spend this last upcoming year for our funding.

A. Specific Aim 3: *to study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance*. We completed during the last year, with work from our current mentored postdoctoral fellow, Michael Topper, Ph.D. and former mentee, Meredith Stone, Ph.D., exciting studies which are now published (*Topper et al, Cell, 2017; Stone et al, PNAS, 2017)* for **Major Task 1**: *to develop the in-vitro pre-clinical systems to outline the sensitivities and derive molecular signatures that track with these*, and **Major Task 2:** *to develop in-vivo pre-clinical systems to outline the potential efficacy of epigenetic therapy sensitization to immunotherapy for targeting checkpoints which drive immune tolerance*. The major findings, as detailed directly below, involve discoveries providing key insight into how epigenetic therapy may help reverse immune evasion to help sensitize to

immune checkpoint therapy for OC, and a growing biomarker system for potentially predicting patient responses and monitoring therapy.

***What was accomplished under these goals*?**

A. Specific Aim 3: Major Task 1: *to develop the in-vitro pre-clinical systems to outline the sensitivities and derive molecular signatures that track with these*. Progress is as follows:

1. *In work published in 2015 (Chiappinelli et al, Cell, 2015),* we defined that, in human OC cells, a cytosolic double stranded RNA (dsRNA) viral defense pathway is a core functional circuit for an AZA induced interferon response and we defined the potential for constituent genes and endogenous retroviral transcripts (HERV's) in this drug response for predicting responses to immune checkpoint therapy (**Fig. 1**). Central to this induction is upregulation of a viral defense pathway which we have functionally studied much further using a mouse model of OC (**Stone et al, PNAS, , 2017**) with important contributions as well from two mouse models of non-small cell lung cancer (NSCLC) (**Topper et al, Cell, 2017**). The data highly support the functionality of the above pathway for driving tumor induced, immune cell attraction, for deriving key biomarker strategies, and for developing new epigenetic therapy approaches for OC.

B. Specific Aim 3: Major Task 2: *to develop in-vivo pre-clinical systems to outline the potential efficacy of epigenetic therapy sensitization to immunotherapy for targeting checkpoints which drive immune tolerance*. In the immediately above mentioned work, as, defined further below, in studies led by Cindy Zahnow and drawing on a paradigm developed by Dr. Topper, a new treatment schema was fully applied to the OC model. Specific accomplishments are:

1. *Deriving, in a mouse model of OC model, a new combinatorial strategy for epigenetic therapy to pair with immune checkpoint therapy*.

As mentioned in the last progress report, and now completed, a graduate student with Dr. Baylin, Michael Topper, in collaboration with our past TEAL award scholars, postdoctoral fellow Kate Chiappinelli (now a faculty member at George Washington) and graduate student, Meredith Stone (now a Postdoctoral Fellow, at the University of Pennsylvania), have all played seminal roles in studying how we can improve a combinatorial, epigenetic therapy paradigm, enhancing the efficacy of immune checkpoint therapy (**Stone et al, PNAS, 2017; Topper et al, Cell, 2017**). The concepts have made much impact nationally and internationally for the possibilities and the approaches are now in trials, including our leveraged trial for advanced OC. A summary of the concepts is in **Fig. 1**. The next couple of years will be pivotal for seeing the ultimate clinical impact of the work for changing cancer management.

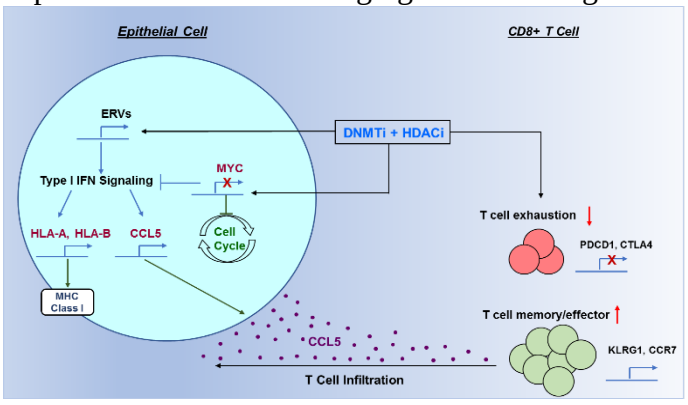

Fig.1. Summary of published data from the graphic for, Topper et al Cell, 2017 and as extrapolated to a model for OC (Stone et al Cell, 2017). The DNMTi plus HDACi treatment results in up-regulation of ERV's and type 1 interferon signaling to result in increased antigen presentation, upregulation of the cytokine, CCL5 accompanying T-cell attraction to tumors, and reversion of T-cell exhaustion to a cell effector state (up-regulation of genes like KLRG1 and CCR7 as shown). A key step is concomitant down-regulation of CMYC signaling which enhances reversion of tumor immune evasion.

2. *Ramifications of the above for future work. As been alluded to earlier extrapolating the above, newly derived epigenetic treatment schema to deeply study the syngeneic mouse model for OC described in our last progress report*. Again, the work has involved great participation from our mentored post-doctoral fellow, Dr. Chiappinelli and a graduate student, Meredith Stone. The model is the MOSE mouse model of serous OC and we have now completed a body of work which is now in press (**Stone et al, PNAS, 2017**). In this model, as we have previously outlined, the mice receive tumor cells intraperitoneally and develop ascites in a manner similar to what can occur in patients with advanced OC. This model is known to be poorly immunogenic giving us the opportunity to determine whether our new epigenetic therapy strategies can alter this scenario and sensitize to immune checkpoint therapy in so doing. The following important data, all in the above in press paper, have emerged:

C. Summary of progress for Specific Aim 3 and majors tasks 1 and 2: As outlined in the sections above, our progress has been extensive in the past year in further understanding how use of epigenetic drugs which inhibit DNA methylation (AZA) and inhibit histone deactylases (HDACi) may be used to increase the efficacy of immune checkpoint therapy in patients with OC. Our new strategy to augment the effects of AZA alone by combining this drug with a deeply researched plan for chronic administration of low dose HDACi's has now been extensively explored over the past years. This importantly, includes fully taking this therapy strategy to in vivo treatment to our mouse MOSE OC model and in combination with immune checkpoint therapy. All of this now shows that the combined epigenetic therapies increase tumor signaling for attraction of activated T-cells and provides for anti-tumor effects with prolongation of survival. These data provide substrate for our honing this treatment regimen further during the next year, and our continuing to explore the implications of all of these studies towards the present leveraged clinical trial for patients with advanced OC and for designing future trials. Finally, Dr. Topper is also concentrating deeply with Dr. Chiappinelli on a deep molecular analysis of multiple OC cell lines treated with the epigenetic therapy paradigms he has developed. The goal of these studies is to outline in novel ways, the upstream mechanisms by which the epigenetic therapy helps reverse tumor evasion including defining upstream signals that drive the entire induced viral defense response.

D. Specific Aim 4: *Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC*: **Major Task 1:** *Follow biochemical hypotheses for designing combinations of the epigenetic drugs used in all studies above with new agents targeting additional steps in chromatin control of gene expression – the goal is to improve reversal of abnormal gene silencing in OC*: These studies, under the direction of Dr. Peter Jones at the Van Andel Research Institute (VARI), with close collaboration from Dr. Baylin have now

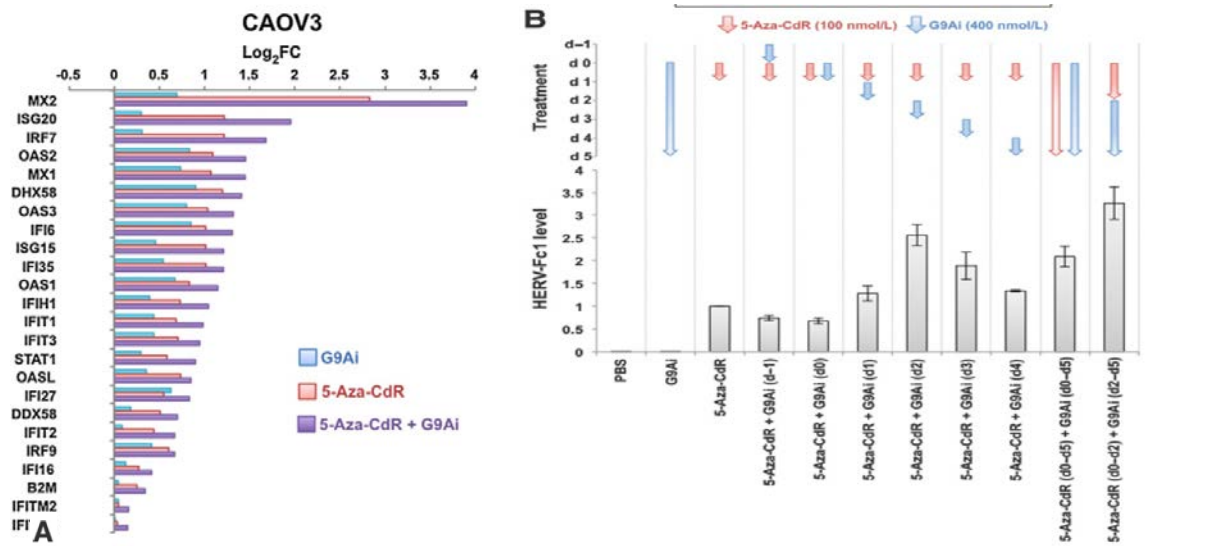

Figure 2. A. Combination treatments further upregulated viral defense genes in A2780 and CAOV3 cells. Bar graphs show the expression fold change (in log2 values) of 24 viral defense genes as per our previously published studies (Rouloix et al, Cell 2015; Chiappinelli et al, Cell, 2015) in CAOV3, ovarian cancer cells after G9Ai, 5-aza-CdR, and combination treatment compared with untreated cells. B. Effects of dosing schedule by combination treatment with G9Ai and 5-aza-CdR on HERV-Fc1 expression. A2780 cells (2.5 x 10^5) were seeded in 100-mm dishes at day 1, then treated with 400 nmol/L G9Ai, 100 nmol/L 5-aza-CdR, or their combinations according to the schedule shown by arrows at the top. Cells were harvested at day 5 after 5-aza-CdR treatment. HERV-Fc1 expression levels were then assayed by quantitative RT-PCR using the expression levels of TBP as a loading control and normalized to the level of HERV-Fc1 expression after 5-aza-CdR treatment alone. Values are presented as mean +/- SEM of three independent experiments. A one-way repeated-measures ANOVA was used for statistical analysis.

been completed and published during this past year (*Liu et al, Cancer Res., 2018*). We have found that, in OC cell lines with high levels of G9A expression, pharmacologic inhibition of G9A, by the G9a inhibitor (G9ai), UNC0638 added to the DNA methylation inhibitor (DNMTi), 5-aza-20- deoxycytidine (5-aza-CdR) AZA, induces synergistic antitumor effects. The drug combination, as analyzed by RNA-seq, synergistically enhances the viral defense signaling effects induced by AZA in ovarian cancer cell lines including upregulation of endogenous retroviruses (ERV) (**Fig. 2**). G9Ai treatment further reduced H3K9me2 levels within the long terminal repeat regions of ERV's, resulting in further increases of ERV expression and enhancing "viral mimicry" effects. In contrast, G9Ai and 5-aza-CdR were not synergistic in cell lines with low basal G9A levels. Taken together, our results suggest that the synergistic effects of combination treatment with DNMTi and G9Ai may serve as a novel therapeutic strategy for patients with ovarian cancer with high levels of G9A expression. Indeed, in TCGA primary cancer samples, G9a is overexpressed compared to multiple normal samples and levels are highest in OC. Importantly, the Baylin lab has shown in the past that G9a, and the H3K9me2 mark is tightly tied to the start sites of genes affected by abnormal DNA methylation in cancer. This mark, and G9A, leave these genes when the abnormal methylation is reversed by AZA, the key drug being used in our studies of OC in the previous sections and in the now ongoing clinical trial.

In summary, addition of an inhibitor of G9a to AZA suggest a potentially new epigenetic therapy combination which may serve as a novel therapeutic strategy for OC patients. Importantly while the G9ai, UNC0638 works only at high, uM levels to specifically inhibit G9a, and is not soluble for in-vivo administration, we are now in a position to receive new drug from the company GBT. We already are working with this drug and can verify that this G9ai works at nM doses to block G9a. We are now about to embark during this last year on *in-vivo* studies for this drug with mice bearing OC tumors, and also with our mouse model of OC as described in sections above. Thus, we will test its combination with AZA in pre-clinical therapy regimens with tumor bearing mice.

What opportunities for training and professional development did the project provide?

The first four years year of this grant have been exceptionally important as discussed in preceding sections in this regard. All of our mentored fellow trainees, including first *Drs. Chiappinelli now a faculty member in the Cancer Center at George Washington University) and Stone, and now Dr. Topper (both postdoctoral fellows at the University of Pennsylvania and Hopkins, respectively),* have benefitted enormously from participation in all of the work outlined in the above sections and are pursuing careers involving development of epigenetic therapies for OC and other cancers. They have the opportunities to become true leaders in this field.

How were the results disseminated to communities of interest?

As outlined last year, over the three years of studies, and outlined below, we have published multiple of our key studies in top journals (Chiappinelli et al, Cell 2015; Topper et al, cell 2017; Stone et al, PNAS, 2017; Liu et al, Caner Research, 2018). Also, as enumerated below, our

trainees and fellows have consistently in top research forums. As outlined in the past progress report, Dr. Chiappinelli's work has been the subject of a review article (Chiappinelli et al, Cancer Research, 2016) and also received an editorial in the New England Journal of Medicine (Dear et al, NEJM, 2016).

What do you plan to do during the next reporting period to accomplish the goals and objectives?

During this last year two key studies will be particularly pursued. First, no matter what the interim results are for our leveraged trial with Dr. Slamon, we will be analyzing pre- and post-biopsy tumor samples from the patients. These studies will teach us how and whether our epigenetic therapy is getting to the tumors and activating the responses we have observed in all of our pre-clinical work throughout this TEAL funding. Second, as described earlier, we will be starting in-vivo mouse studies with our new G9a inhibitor from GBT to try to develop a new epigenetic treatment strategy for advanced OC.

4. IMPACT

***What was the impact on the development of the principal discipline(s) of the project*?**

Continuing over this past year, and throughout our funding period, our work has brought the PI, his collaborators, and all trainees ever deeper into studying biology of serous OC and studies to for develop new means to treat this disease. All of us have been widely asked to lecture at national and international meetings specifically regarding the potential for epigenetic therapy to increase the efficacy of immune checkpoint therapy and for providing insight into the mechanisms that may be involved. As per sections above, the trainees and faculty involved with the TEAL have also been called upon for many lectures at OC specialty meetings and general cancer research meetings. Finally, the paradigms we have developed for potential treatment strategies for OC have generated tremendous national and international attention. Trials based on our work, including new strategies for generating the viral mimicry mechanisms we first reported are ongoing throughout the world. As has been true throughout our funding period, our great hope is that, at the end of the day, our studies will lead new therapy regimens for OC and other major cancer types to the benefit of a great number of patients.

***What was the impact on other disciplines*?**

As outlined just above, our basic, pre-clinical studies of OC have had widespread impact. The new combination regimen of AZA plus HDACi's, given with immune checkpoint therapy, as defined in the in our Cell and PNAS papers have generated lab studies and clinical trials not only for OC but lung, colon, pancreatic, breast and other cancers. These include major studies in our Stand up to Cancer (SU2C) epigenetic therapy team co-led by Drs. Baylin and Jones. All of our trials, and most of the trials by others alluded to above, intense pre- and post-treatment battery of correlative science studies based on our work with the aim of developing predictive biomarkers for therapy outcomes including drug effects on immune cells. Emphasis on the latter is a new result which our studies in Cell and PNAS have helped fuel.

***What was the impact on technology transfer*?**

As outlined in last year's report, and now augmented by the work over the past year, our viral defense signature, inclusive of the ERV transcripts has a patent applied for status as a biomarker system to predict and monitor the efficacy of applying epigenetic therapy to sensitize patients with advanced OC and all cancer types, to immune checkpoint therapy. The work in the ongoing,

leveraged OC trial with AZA plus immune checkpoint therapy, and new trials for NSCLC and bladder cancer, funded through Merck and Genetech Catalyst Awards to our above SU2C team may hopefully help Hopkins push forward the potential to license the above biomarker signature.

***What was the impact on society beyond science and technology*?**

Hopefully, as outlined above, the biggest impact of our studies will be for patients. As also mentioned above, new trials are now testing our therapy paradigms for sensitizing to immune checkpoint therapy in patients with advanced NSCLC and bladder cancer and any therapy efficacies observed could provide the greatest impact we could seek for our work including for technologies to pursue the biomarkers discussed above.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

At present, we do not anticipate any major changes to our work scope and directions. We will continue to focus on Specific Aims 3 and 4 as defined earlier and trying to maximize our epigenetic therapies for OC including continued new work with the G9ai to develop in-vivo pre-clinical studies during the coming year (Specific Aim 4).

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

None anticipated at this time. We have been moving at a rapid pace during the past year and hope to continue this.

Changes that had a significant impact on expenditures

None anticipated at this time.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

None anticipated at this time.

6. PRODUCTS

Publications, conference papers and presentations

Publications

Liu M, Thomas SL, DeWitt AK, Zhou W, Madaj ZB, Ohtani H, Baylin SB, Liang G and Jones PA. Dual inhibition of DNA and histone methyltransferases increases viral mimicry in ovarian cancer cells. Cancer Research 78(20): 5754-5766, 2018.

Presentations

Stephen B. Baylin, M.D.

10/2017	2nd BNMC Translational Genomics and Epigenomics Symposium, Roswell Park Cancer Institute, Buffalo, NY
10/2017	Mayo Civic Center, Rochester, MN

10/2017	Abbvie Celebration of Science, Chicago, IL
11/2017	Miriam and Sheldon G. Adelson Medical Research Foundation meeting, Las Vegas, NV
11/2017	Mirati SAB, Boston, MA
12/2017	ASH Annual Mtg, Atlanta, GA
12/2017	DOD Oral Presentation, Washington, DC
1/2018	Joint RSM and Pathological Society Winter Mtg., London, UK
1/2018	SU2C Scientific Summit, Santa Monica, CA
2/2018	Keystone, Denver, CO
3/2018	AACR, Atlanta
3/2018	ADELIH Congress 3rd Annual Mtg., Paris, France
4/2018	AACR Annual Mtg., Chicago, IL
4/2018	National Academy of Science Induction, Washington, DC
5/2018	Cancer Epigenetic Distinguished Lecture Series, MD Anderson Cancer Center, Houston, TX
5/2018	Miriam and Sheldon G. Adelson Medical Research Foundation meeting, Las Vegas, NV
7/2018	Cancer Epigenetic Therapies, Naples, Italy
7/2018	VARI Summer Retreat, Grand Rapids, MI
9/2018	ISREC-SCCL Symposium, Lausaune
9/2018	Forbeck Focus Mtg., Geneva National Resort, Lake Geneva, WI
9/2018	SU2C Telecast, Santa Monica, CA

Cynthia A. Zahnow, Ph.D.

12/2017	Invited Speaker, “Epigenetic Regulation of the Immune Microenvironment” San Antonio Breast Cancer Symposium, San Antonio Texas
9/2018	Invited Speaker, AACR International Conference on Translational Cancer Medicine being held in cooperation with the Latin American Cooperative Oncology Group (LACOG)

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

Individuals who have worked on the project

Johns Hopkins University

Name:	Stephen B. Baylin, M.D.
Project Role:	PI (Senior/Key Personnel)
Research Identifier:	N/A
Nearest person month worked:	4
Contribution to Project:	Dr. Baylin oversees all studies and activities conducted under this proposal.
Funding Support:	See Other Support

Name:	Cynthia Zahnow, Ph.D.
Project Role:	Co-Investigator (Senior/Key Personnel)
Research Identifier:	N/A
Nearest person month worked:	2
Contribution to Project:	Dr. Zahnow collaborates with Dr. Baylin on all of the studies in the lab.
Funding Support:	See Other Support

Name:	Drew Pardoll, M.D., Ph.D.
Project Role:	Co-Investigator (Senior/Key Personnel)
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Pardoll works with the Baylin group for all of the studies on how epigenetic therapy can sensitize ovarian cancers to immune checkpoint therapy.
Funding Support:	See Other Support

Name:	Ray-Whay Yen
Project Role:	Research Associate
Research Identifier:	N/A
Nearest person month worked:	8
Contribution to Project:	Ms. Yen is responsible for working with the entire Hopkins group for all of the pre-clinical work on ovarian cancer.
Funding Support:	No change

Name:	Michael Topper, Ph.D.
Project Role:	Postdoctoral Fellow / Teal Junior Scientist
Research Identifier:	N/A
Nearest person month worked:	9
Contribution to Project:	As outlined above, Dr. Topper continues to contribute enormously to our Teal work and former trainee Dr. Chiappinelli also continues collaborating with him. His academic growth is discussed in detail in Section 8, Special Reporting.
Funding Support:	Dr. Topper became a Postdoctoral Fellow on 10/21/17. He is supported 75% on the DOD Teal, 20% on an

Emerson award, and 5% on an American Lung Association 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?

Yes. See next pages for Drs. Baylin, Zahnow and Pardoll's Other Support.

OTHER SUPPORT

BAYLIN, STEPHEN B.

ACTIVE

P30 CA006973 (PI: Nelson)
Title: Regional Oncology Research Center – Senior Leader
Time Commitment: 0.6 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Precilla L. Belin
Address of Grants Officer: National Cancer Institute, Building 6116-700, 6116 Executive Blvd, Rockville, MD 20852
Performance Period: 5/7/1997-4/30/2022
Level of Funding:
Project's Goal(s): CORE grant for the Johns Hopkins Oncology Center. Stephen Baylin receives salary support only for leadership and microarray core responsibilities.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

R01 ES011858 (PI: Baylin)
Title: DNA Methyltransferase Gene Expression in Colon Cancer
Time Commitment: 1.44 calendar
Supporting Agency: NIH/NIEHS
Procuring Contracting/Grants Officer: Frederick Tyson
Address of Grants Officer: National Institute of Health, Keystone Park 3064, 615 Davis Dr, Durham, NC 27709
Performance Period: 4/1/1991-5/31/2019
Level of Funding:
Project's Goal(s): Understand, further, the role of altered regulation and patterns of DNA methylation in the progression of colon cancer.
Specific Aims: 1. To determine mechanisms by which SOX17 blocks Wnt activation in CRC evolution. 2. To develop mouse models for CRC evolution based on epigenetic loss of Hic1. 3. To explore specific stages of CRC tumorigenesis mediated by epigenetic silencing of stem/progenitor cell related genes. 4. To define molecular determinants which initiate and/or maintain gene promoter DNA hypermethylation and gene silencing in CRC evolution.
Project Overlap or Parallel: No scientific or budgetary overlap.

90046519 (PI: Baylin/Casero/Zahnow)
Title: Novel Therapies Targeting Epigenetic Silencing of Tumor Suppressors
Time Commitment: 0.06 calendar
Supporting Agency: Samuel Waxman Cancer Research Foundation
Procuring Contracting/Grants Officer: Carole Asher
Address of Grants Officer: 420 Lexington Ave., Suite 825, New York, NY 10170
Performance Period: 7/1/2011-6/30/2019
Level of Funding:
Project's Goal(s): The goals of this project are: Project 1: To examine newly identified lysine specific demethylase 1 (LSD1) inhibitors in order to advance the understanding of the functioning and targeting of LSD1 for clinical utility. Project 2: To show that epigenetic therapy at very low, non-toxic doses, can dramatically blunt the tumorigenic properties of subpopulations of leukemic and solid tumor populations of "stem-like" cells. Project 3: To demonstrate that low dose epigenetic therapy re-sensitizes drug tolerant breast cancer cells to conventional, single agent chemotherapeutics or targeted therapy.

Specific Aims: 1. To perform, in Kasumi AML cells, and other lines, genome-wide studies of DNA methylation, chromatin and, gene expression patterns, including pathway analyses, for activating and repressive marks in separated populations of tumorigenic CD34+/CD38- versus non-tumorigenic CD34- cells. 2. To examine changes in the above genome-wide patterns induced by low doses of DNA demethylating and histone deacetylation inhibiting drugs, already shown to inhibit the leukemic engraftment of the whole cell population, alone and together, on the above separated populations. 3. To derive markers for prediction and monitoring of epigenetic therapy from the above studies and which can be studied in primary tumor samples, and patient samples.
Project Overlap or Parallel: No scientific or budgetary overlap.

90075114 (PI: Baylin)
Title: Bringing Epigenetic Therapy to the Management of Ovarian and Other Cancers
Time Commitment: 1.8 calendar
Supporting Agency: Miriam & Sheldon Adelson Medical Research Foundation
Procuring Contracting/Grants Officer: Joseph Bigley
Address of Grants Officer: OncoMethylome Sciences, 2505 Meridian Parkway, Suite 310, Durham, NC 27713
Performance Period: 10/1/2017-9/30/2019
Level of Funding:
Project's Goal(s): We are embarked on in-depth pre-clinical studies designed to directly bring "epigenetic" therapy, using existing DNA de-methylating agents and histone deactylase inhibitors (HDACi's), to the therapeutic management of advanced ovarian and other cancers.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

90075113 (PI: Velculescu)
Title: Ovarian Cancer Genome Analysis Platform
Time Commitment: 0.36 calendar
Supporting Agency: Miriam & Sheldon Adelson Medical Research Foundation
Procuring Contracting/Grants Officer: Joseph Bigley
Address of Grants Officer: OncoMethylome Sciences, 2505 Meridian Parkway, Suite 310, Durham, NC 27713
Performance Period: 10/1/2017-9/30/2019
Level of Funding:
Project's Goal(s): N/A
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

W81XWH-14-1-0385 (Baylin)
Title: A New Paradigm for the Treatment of Ovarian Cancer: The Use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy
Effort: 3 calendar
Supporting Agency: CDMRP
Name of Procuring Contracting/Grants Officer: Susan Dellinger, Grants Officer
Address of Funding Agency: 1077 Patchel St., Bldg 1077, Fort Detrick, MD 21702
Period of Performance: 09/30/2014-09/29/2019
Level of Funding:
Project's Goal: The major goal of this project is to robustly prolong the survival of patients with serous ovarian cancer (OC) through introducing epigenetic therapy paradigms
Specific Aims: 1) To uncover the mechanisms through which epigenetic therapy may, alone, achieve robust, durable responses in patients with advanced ovarian cancer (OC) 2) Study how epigenetic therapy may sensitize OC cells to subsequent chemotherapies 3) Study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune

tolerance 4) Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC 5) Bring all of the above studies to bear on leveraging clinical trials of epigenetic therapy on OC
Project Overlap or Parallel: No scientific or budgetary overlap.

90058334 (PI: Baylin)
Title: Clinical trials of epigenetic therapy sensitized patients with advanced non-small cell lung cancer to chemotherapy and immunotherapy
Time Commitment: 0.06 calendar
Supporting Agency: AACR – Jim Toth Sr. Breakthrough Prize in Lung Cancer
Procuring Contracting/Grants Officer: Susan Frank
Address of Grants Officer: 1900 Avenue of the Stars, 14th Floor, Los Angeles, CA 90067
Performance Period: 7/1/2014-6/30/2019
Level of Funding:
Project's Goal(s): Enroll the targeted number of patients and complete both clinical trials which, if efficacy emerges, could truly lead to a new, robust management of advanced NSCLC, the world's biggest cancer killer.
Specific Aims: Milestone 1: This phase 2 trial for patients with NSCLC to directly assess priming of epigenetic therapy to subsequent chemotherapy is now open and accruing. Milestone 2: This phase 2 trial for patients with NSCLC, to directly assess priming of epigenetic therapy for anti-PD1 immuno-therapy, is now open and accruing at Hopkins – and being sent to IRB's at USC amd Memorial Sloan Kettering.
Project Overlap or Parallel: No scientific or budgetary overlap.

90061408 (PI: Baylin)
Title: Clinical trials of epigenetic therapy in non-small cell lung cancer
Time Commitment: 0.06 calendar
Supporting Agency: Rising Tide Foundation
Procuring Contracting/Grants Officer: Eveline Mumenthaler
Address of Grants Officer: Herrenacker 15, 8200 Schaffhausen, Switzerland
Performance Period: 1/1/2015-12/31/2018
Level of Funding:
Project's Goal(s): We are addressing the hypothesis that reversal of cancer-specific DNA methylation and chromatin abnormalities can potently change the management of NSCLC.
Specific Aims: The two new trials, scheduled to involve a total of some 315 patients, are deigned to formally test: 1) whether low dose epigenetic therapy with a DNA demethylating agent plus a histone deactylase inhibitor does sensitize patients with advanced NSCLC to subsequent, standard, chemotherapy; and 2) whether this treatment sensitizes the same patient population to immunotherapy targeting immune checkpoints which drive immune tolerance.
Project Overlap or Parallel: No scientific or budgetary overlap.

R01 HD082098 (PI: Zambidis)
Title: Functional vascular progenitors from naïve human iPSC
Time Commitment: 0.24 calendar
Supporting Agency: Natl Ins of Child Health & Human
Procuring Contracting/Grants Officer: Mahua Mukhopadhyay
Address of Grants Officer: P.O. Box 3006, Rockville, MD 20847
Performance Period: 5/1/2015-2/29/2020
Level of Funding:

Project's Goal(s): To develop novel gene targeting and regeneration approaches for treating pediatric and adult vascular disorders using a newly discovered class of human iPSC converted to a ground state of naïve pluripotency.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

90061607 (PI: Baylin)
Title: VARI-SU2C Epigenetics Dream Team
Time Commitment: 0.06 calendar
Supporting Agency: Van Andel Research Institute
Procuring Contracting/Grants Officer: Jerry Callahan
Address of Grants Officer: 333 Bostwick Avenue, NE, Grand Rapids, MI 49503
Performance Period: 10/1/2014-9/30/2020
Level of Funding:
Project's Goal(s): Our Dream Team unites scientists at major cancer research institutions who are poised to propel the early promise of epigenetic therapy in blood malignancies to the forefront of management for patients with breast, colon and lung cancer.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

90067293 (PI: Baylin)
Title: Understanding the mechanisms underlying how epigenetic therapy may sensitize patients with multiple human cancer types to immune checkpoint therapy
Time Commitment: 0.12 calendar
Supporting Agency: Janssen Research & Development LLC
Procuring Contracting/Grants Officer: Dashyant Dhanah
Address of Grants Officer: 1400 McKean Rd., Spring House, PA 19477
Performance Period: 2/26/2016-2/26/2019
Level of Funding:
Project's Goal(s): A collaboration between Janssen Res & Dev and The Cancer Center at Johns Hopkins to study mechanisms underlying how epigenetic therapy may sensitize patients with multiple human cancer types to immune checkpoint therapy.
Specific Aims: To continue investigating mechanisms in tumor cells which may underlie epigenetic therapy priming to immune checkpoint therapy and provide biomarker strategies for prediction of efficacy.
Project Overlap or Parallel: No scientific or budgetary overlap.

90074377 (PI: Baylin)
Title: Developing a lab model for lung cancer initiation, risk and prevention
Time Commitment: 0.36 calendar
Supporting Agency: Am Lung Association
Procuring Contracting/Grants Officer: Alexandra Sierra
Address of Grants Officer: 55 West Wacker Dr., Suite 1150, Chicago, IL 60601
Performance Period: 7/1/2017-6/30/2019
Level of Funding:
Project's Goal(s): Our preliminary data and proposed studies for this proposal aim to use a new approach to study, in human lung epithelial cells *in-vitro*, the consequences of chronic exposure to low doses of cigarette smoke extract (CSC).
Specific Aims: Specific Aim 1: Determine effects of CSC exposure on epigenetic alterations and their ability to promote subsequent sensitization to transformation in cultured primary human bronchial epithelial cells and normal lung organoid cultures. Specific Aim 2: To determine cancer-driver roles

for genes with CSC-induced abnormal epigenetic events using the CRISPR-based gene manipulation approach.
Project Overlap or Parallel: No scientific or budgetary overlap.

R21 CA212495 (PI: Easwaran)
Title: High-efficiency microfluidic-assisted single-cell DNA methylome sequencing
Time Commitment: 0.24 calendar
Supporting Agency: NCI
Procuring Contracting/Grants Officer: Jerry Li
Address of Grants Officer: 9609 Medical Center Dr., Bethesda, MD 20892
Performance Period: 8/3/2017-7/30/2020
Level of Funding:
Project's Goal(s): The goal of this project is to devise a novel methodology for high-efficiency multiplexed profiling of DNA methylation in single cells by bisulfite sequencing.
Specific Aims: Aim-1: Develop a novel methodology that combines DNA isolation and bisulfite treatment with microfluidics-assisted modified genome amplification and library preparation from single cells. Aim-2: Validate methodology by performing WGBS on few cell numbers and single cells. Aim 3: Demonstrate 'utility of technology' by mapping progressive epigenetic changes in ex vivo organoid models of tumorigenesis.
Project Overlap or Parallel: No scientific or budgetary overlap.

AWARDED SINCE LAST SUBMISSION

90076072 (PI: Anagnostou)
Title: Genomic and Epigenetic Mechanisms of Response and Resistance to Epigenetic….
Time Commitment: 0.6 calendar
Supporting Agency: V Foundation for Cancer Research
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: 14600 Weston Parkway, Cary, NC 27513
Performance Period: 11/1/2017-11/1/2020
Level of Funding:
Project's Goal(s): We propose to pinpoint the mechanisms that mediate response and resistance to these therapies by looking at the genetic make-up of cancer cells as well as by studying the tumor microenvironment.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

90077253 (PI: Baylin)
Title: Effect of GBT G9a inhibitors in cancer cell lines and in xenograft models
Time Commitment: 0.12 calendar
Supporting Agency: Global Blood Therapeutics
Procuring Contracting/Grants Officer: Tony Peng
Address of Grants Officer: 171 Oyster Point Blvd, Ste. 300, South San Francisco, CA 94080
Performance Period: 2/26/2018-2/25/2020
Level of Funding:
Project's Goal(s): The ultimate goal is to translate all promising data into clinical trials for cancer management.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

900780913 (PI: Baylin)
Title: Janssen Initiative For Determining Colon Cancer Risk and Exploring means For Prevention and Interception
Time Commitment: 0.6 calendar
Supporting Agency: Janssen Research & Development LLC
Procuring Contracting/Grants Officer: Dashyant Dhanah
Address of Grants Officer: 1400 McKean Rd., Spring House, PA 19477
Performance Period: 4/10/2018-4/01/2021
Level of Funding:
Project's Goal(s): Our group proposes to participate in this important initiative by exploring how the epigenetic abnormalities in cancer, long the focus of our group, contribute to the risk for and initiation and early progression of colorectal cancer (CRC).
Specific Aims: Specific Aim 1: Continuing pre-clinical studies in colon organoids aimed at outlining the dynamics through which epigenetic abnormalities contribute to the risk, initiation, and progression of CRC. Specific Aim 2: Collaborative analysis with Janssen of bioinformatics data currently derived by Janssen and Hopkins scientists. Specific Aim 3: Developing from the studies in Aims 1 and 2, and testing in a prospective manner in key CRC risk and established disease cohorts, the efficacies of derived biomarker strategies.
Project Overlap or Parallel: No scientific or budgetary overlap.

90078135 (PI: Baylin)
Title: The Celgene Cancer Center Consortium: Targeting UHRF1 to Cancer Therapy
Time Commitment: 0.6 calendar
Supporting Agency: Celgene
Procuring Contracting/Grants Officer: Kevin Mello
Address of Grants Officer: 86 Morris Ave., Summit, NJ 07901
Performance Period: 3/13/2018-3/21/2021
Level of Funding:
Project's Goal(s): We anticipate this will be a collaborative effort. Our hope is that each institution provides the biology, translational tools and strategy with our input to these areas.
Specific Aims: Our aim is to collaboratively develop for cancer therapy, inhibitors of UHRF1, a protein essential in mammalian cells for targeting DNA methyltransferases (DNMT's) to DNA to maintain DNA methylation.
Project Overlap or Parallel: No scientific or budgetary overlap.

90077253 (PI: Baylin)
Title: Effect of GBT G9a inhibitors in cancer cell lines and in xenografts models
Time Commitment: 0.12 calendar
Supporting Agency: Global Blood Therapeutics
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: 171 Oyster Point Blvd., South San Francisco, CA 94080
Performance Period: 2/26/2018-2/25/2020
Level of Funding:
Project's Goal(s): Unknown
Specific Aims: Unknown
Project Overlap or Parallel: No scientific or budgetary overlap.

90078168 (PI: Baylin)
Title: Developing a neo-antigen based anti-lung cancer vaccine for use with combination epigenetic-immunotherapy
Time Commitment: 0.24 calendar
Supporting Agency: Emerson

Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 6/01/2018-5/31/2020
Level of Funding:
Project's Goal(s): We will mine exome- and RNA-seq data from pre-and post biopsies from our ongoing SU2C trial.
Specific Aims: Specific Aim #1: To determine the clonal expansion dynamics of memory and effector tumor associated CD8+ T cell populations in response to combination epigenetic-immunotherapy. Specific Aim#2: To identify therapy induced tumor antigen specific CD8+ T cells and develop a pooled peptide vaccination to deploy with combination epigenetic-immunotherapy.
Project Overlap or Parallel: No scientific or budgetary overlap.

R01 CA230995 (PI: Easwaran/Baylin)
Title: (PQ4) - Tools for simultaneous disruption of multiple epigenetically silenced genes for studying their roles in tumorigenesis using ex vivo human and mouse colon organoid and in vivo mouse models
Time Commitment: 1.08 calendar
Supporting Agency: NIH
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 9/1/2018-8/31/2023
Level of Funding:
Project's Goal(s): This proposal will develop generic tools for simultaneous manipulation of multiple genes in human cancer-relevant models that can be easily translated to other cancer models.
Specific Aims: Aim-1: Develop tools for inactivation of multiple gene combinations and identify effects on early tumor development using colon organoid model. Aim-2: Determine whether, and how, inactivation of multiple chosen genes promotes tumorigenesis by key colorectal cancer driver mutations. Aim-3: In vivo modeling to identify roles of cancer-specific epigenetically silenced genes in initiating in situ tumorigenesis.
Project Overlap or Parallel: No scientific or budgetary overlap.

COMPLETED SINCE LAST SUBMISSION

90061810 (PI: Yegnasubramanian)
Title: Enhancing Prostate Cancer Immunotherapy Through Epigenetic Reprogramming
Time Commitment: 0.6 calendar
Supporting Agency: Prostate Cancer Foundation
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 12/24/14-12/24/17
Level of Funding:
Project's Goal(s): The main goal of this project is to test the hypothesis that epigenetic therapy plus immunotherapy can be effective in the control of advanced prostate cancer.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

W81XWH-13-1-0199 (PI: Chan/Baylin)
Title: Targeting Master Regulators of the Breast Cancer Metastasis Transcriptome
Time Commitment: 0.12 calendar
Supporting Agency: Memorial Sloan-Kettering Cancer Center
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 7/1/2013-6/30/2018

Level of Funding:
Project's Goal(s): The Baylin lab will help perform CHIP seq and help analyze the chromatin state data for both the isogenic cell line systems that model differential metastatic ability.
Specific Aims: Aim 1: N/A
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

R01 CA185357 (PI: Ahuja)
Title: (PQD3) Molecular Profiles associated with Long-Term Survival in pancreas Cancer
Time Commitment: 0.24 calendar
Supporting Agency: NCI
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 6/1/2014-5/30/2018
Level of Funding:
Project's Goal(s): Identify genomic and epigenomic signatures of pancreas cancer patients who have long-term survival using a large dataset.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Brahmer/Baylin)
Title: Viral Defense Gene Expression Patterns and Response to Immune Checkpoint Blockade in NSCLC
Time Commitment: 0.12 calendar
Supporting Agency: Bristol Myers Squibb
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 8/14/2015-4/14/2018
Level of Funding:
Project's Goal(s): Unknown
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

OTHER SUPPORT

ZAHNOW, CYNTHIA A.

ACTIVE

90046519 (PI: Casero/Baylin/Zahnow)
Title: Novel therapies targeting epigenetic silencing of tumor suppressors
Time Commitment: .12 calendar
Supporting Agency: Samuel Waxman Cancer Research Foundation
Procuring Contracting/Grants Officer: Carole Asher
Address of Grants Officer: 420 Lexington Ave., Suite 825, New York, NY 10170
Performance Period: 7/1/2011-6/30/2019
Level of Funding:
Project's Goal(s): The goals of Dr. Zahnow's project within this Collaborative Grant is to demonstrate that low dose epigenetic therapy re-sensitizes drug tolerant breast cancer cells to conventional, single agent chemotherapeutics or targeted therapy.
Specific Aims: 1. To test whether Azacytidine can sensitize endocrine-resistant breast cancers to anti-estrogen therapy. 2. To continue our investigation of the role of the immune system in the anti-tumorigenic response of breast cancer cells to epigenetic therapy with a special focus on interferon signaling and activation. **Justification:** This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

P30 CA006973 (PI: Nelson)
Title: Regional Oncology Research Center – Resource Director
Time Commitment: 4.5 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Devi Vembu
Address of Grants Officer: National Cancer Institute, Building 6116-700, 6116 Executive Blvd, Rockville, MD 20852
Performance Period: 5/7/1997-4/30/2022
Level of Funding:
Project's Goal(s): CORE grant for the Johns Hopkins Oncology Center. Dr. Zahnow receives salary support only for serving as the Director of the Animal Facility and administrative duties to the Oncology Center.
Specific Aims: N/A
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

Award ID: W81XWH-14-1-0385(Baylin)
Title: A New Paradigm for the Treatment of Ovarian Cancer: The Use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy
Effort: 1.8 calendar
Supporting Agency: CDMRP
Name of Procuring Contracting/Grants Officer: Susan Dellinger, Grants Officer
Address of Funding Agency: 1077 Patchel St., Bldg 1077, Fort Detrick, MD 21702
Period of Performance: 09/30/2014-09/29/2019
Level of Funding:
Project's Goal: The major goal of this project is to robustly prolong the survival of patients with serous ovarian cancer (OC) through introducing epigenetic therapy paradigms
Specific Aims: 1) To uncover the mechanisms through which epigenetic therapy may, alone, achieve robust, durable responses in patients with advanced ovarian cancer (OC), 2) Study

how epigenetic therapy may sensitize OC cells to subsequent chemotherapies, 3) Study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance, 4) Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC, 5) Bring all of the above studies to bear on leveraging clinical trials of epigenetic therapy on OC.
Role: PI
Overlap: None

90075114 (PI: Baylin)
Title: Bringing Epigenetic Therapy to the Management of Ovarian and Other Cancers
Time Commitment: 3 calendar
Supporting Agency: Miriam & Sheldon Adelson Medical Research Foundation
Procuring Contracting/Grants Officer: Marissa White
Address of Grants Officer: 300 First Avenue, Suite 330, Needham, MA 02494
Performance Period: 10/01/2014-9/30/2019
Level of Funding:
Project Goals: We are embarked on in-depth pre-clinical studies designed to directly bring "epigenetic" therapy, using existing DNA de-methylating agents and histone deactylase inhibitors (HDACi's), to the therapeutic management of advanced ovarian and other cancers.
Specific Aims: N/A
Justification: This grant has no overlap with the current proposal.
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Baylin)
Title: Clinical trials of epigenetic therapy in non-small cell lung cancer
Time Commitment: 0.3 calendar
Supporting Agency: Rising Tide Foundation
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 1/1/2015-12/31/2018
Level of Funding:
Project's Goal(s): We are addressing the hypothesis that reversal of cancer-specific DNA methylation and chromatin abnormalities can potently change the management of NSCLC.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

90067293 (PI: Baylin)
Title: Understanding the mechanisms underlying how epigenetic therapy may sensitize patients with multiple human cancer types to immune checkpoint therapy
Time Commitment: .12 calendar
Supporting Agency: Janssen Research & Development LLC
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 2/26/2016-2/25/2019
Level of Funding:
Project's Goal(s): A collaboration between Janssen Res & Dev and The Cancer Center at Johns Hopkins to study mechanisms underlying how epigenetic therapy may sensitize patients with multiple human cancer types to immune checkpoint therapy.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

R01 CA204555 (PI: Sharma)
Title: Evaluation of molecular determinants of racial disparity in triple-negative breast cancer
Time Commitment: 0.48 calendar
Supporting Agency: NIH/NCI

Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 4/1/2017-3/31/2022
Level of Funding:
Project's Goal(s): Our studies focus on examining the key molecules involved in racial disparity in triple negative breast cancer focusing on the role and importance of loss of tumor suppressor genes and resulting 'oncogene addiction' in triple negative breast cancer growth and progression.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

(PI: Shih)
Title: Development of Targeted Therapies for Recurrent Ovarian Cancer
Time Commitment: 0.6 calendar
Supporting Agency: Ovarian Cancer Research Fund
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 1/1/2017-12/31/2019
Level of Funding:
Project's Goal(s): Unknown
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

AWARDED SINCE LAST SUBMISSION

Not applicable

COMPLETED SINCE LAST SUBMISSION

R01 CA184165 (PI: Pandey)
Title: Personalized Therapy of Hormone Refractory Breast Cancer
Time Commitment: 0.6 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Unknown
Address of Grants Officer: Unknown
Performance Period: 4/1/2015-8/19/2018
Level of Funding:
Project's Goal(s): Unknown
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

OTHER SUPPORT

PARDOLL, DREW M.

ACTIVE

Award ID: P50CA098252 (Wu)
Title: SPORE in Cervical Cancer
Effort: 0.24 calendar months
Supporting Agency: NIH/NCI
Name of Procuring Contracting/Grants Officer: Jason Gill
Address of Funding Agency: 9609 Medical Center Drive, Rockville, MD 20850
Performance Period: 09/01/04 – 08/31/19
Level of Funding:
Project's Goal: The development research program role is to identify and select pilot projects with potential for development into full- fledged translational research avenues, collaborations, and new methodologies for integration into other research projects based on the described review criteria.
Specific Aims: 1) Provide initiating funds for novel explorations related to cervical cancer. 2) Integrate the awardee into the SPORE community by participation in monthly meetings, group communications, and opportunities for expanded funding and for collaborations. 3) Review progress and recommend avenues for continuation of successful projects
Role: Co- Director, Developmental Research Program
Overlap: None

Award ID: P30CA06973 (Nelson)
Title: Regional Oncology Research Center
Effort: 0.36 calendar months
Supporting Agency: NIH/NCI
Name of Procuring Contracting/Grants Officer: Jason Gill
Address of funding agency: 9609 Medical Center Drive, Rockville, MD 20850
Performance Period: 08/09/2012-04/30/2022
Level of Funding:
Project's Goal: The major goal of this project is to support research programs and shared resources at the National Cancer Institute Designated Cancer Center. The central goal of the Cancer Immunology program is the development of new effective cancer immunotherapies that are based on understanding the molecular recognition and regulation.
Specific Aims: N/A
Role: Co-Program Leader for Cancer Immunology
Overlap: None

Award ID: N/A (Pardoll)
Title: Analysis of novel immunomodulatory ligands and receptors
Effort: .6 calendar months
Supporting Agency: Compugen Ltd.
Name of Procuring Contracting/Grants Officer: Anat Cohen-Dayag, Ph.D
Address of Funding Agency: 72 Pichas Rosen St., Tel Aviv 69512, Israel
Period of Performance: 01/01/2015-12/31/2019
Level of Funding:
Project's Goal: The major goal of this project is to study the immunobiology and cancer immunotherapy relevance of multiple novel gene products identified as potentially immunomodulatory
Specific Aims: 1) Determine in-house phage display vs conventional hybridoma depending on level of conservation of molecule across species. 2) Expression studies in mice and humans-define target's expression tumor components of the TME, sorted cell populations, purified tumor infiltrates, myeloid and lymphocyte

human-on selected targets. 3) In vitro testing of murine and human antibodies and Fc fusion molecules 4)Antibody/Recombinant Fc fusion experiments with emphasis on antibodies 5) Therapeutic synergy experiments
Role: PI
Overlap: None

Award ID: W81XWH-14-1-0385(Baylin)
Title: A New Paradigm for the Treatment of Ovarian Cancer: The Use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy
Effort: .36 cal months
Supporting Agency: CDMRP
Name of Procuring Contracting/Grants Officer: Susan Dellinger, Grants Officer
Address of Funding Agency: 1077 Patchel St., Bldg 1077, Fort Detrick, MD 21702
Period of Performance: 09/30/2014-09/29/2019
Level of Funding:
Project's Goal: The major goal of this project is to robustly prolong the survival of patients with serous ovarian cancer (OC) through introducing epigenetic therapy paradigms
Specific Aims: 1) To uncover the mechanisms through which epigenetic therapy may, alone, achieve robust, durable responses in patients with advanced ovarian cancer (OC) 2) Study how epigenetic therapy may sensitize OC cells to subsequent chemotherapies 3) Study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance 4) Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC
5) Bring all of the above studies to bear on leveraging clinical trials of epigenetic therapy on OC
Role: Co-Investigator
Overlap: None

Award ID: CA-209-358 (Topalian)
Title: Analysis of PD-1 Blockade in Virus-Associated Cancers on CA-209-358
Effort: .12 Calendar months
Supporting Agency: Bristol Myers Squibb Co
Name of Procuring Contracting/Grants Officer: Les Enterline
Address of Funding Agency: Route 206 and Providence Line Road, Princeton, NJ 08543
Period of Performance: 07/01/2016-12/31/2018
Level of Funding:
Projects Goal: The goal of this project is to characterize changes in the tumor immune microenvironment in pre/post therapy biopsies from patients with advanced virus-associated cancers receiving anti-PD-1 therapy on clinical trial CA209-358, in order to understand response and resistance to therapy.
Specific Aims: N/A
Role: PI
Overlap: None

Award ID: R01CA142779-06A1 (Pardoll/Topalian/Taube)
Title: B7-H1/PD1 modulation in cancer therapy
Effort: 1.2 Calendar months
Supporting Agency: NIH/NCI
Name of Procuring Contracting/Grants Officer: Jacquelyn Saval
Address of Grants Officer: 9609 Medical Center Drive, Rockville, MD 20850
Period of Performance: 12/01/2015-11/30/2020
Level of Funding:
Project's Goal: The major goals of this multi-PI project are to define mechanisms regulating the expression of B7-H1 (PD-L1) by tumor cells and PD-1 by tumor-specific T cells, and to explore the

molecular and immunological mechanisms contributing to the clinical effects of B7-H1/PD-1 blockade in therapeutic trials for patients with advanced metastatic cancers
Specific Aims: 1) Define mechanisms regulating PD-L1 expression by tumor cells and other cell types in the tumor microenvironment 2) Characterize factors influencing PD-1 expression by T cells. 3) Characterize immunological mechanisms underlying the clinical effects of PD-L1/PD-1 blockade in cancer therapy, including the co-expression of multiple checkpoint pathways that might provide resistance pathways to therapy
Overlap: None
Role: MPI (Contact PI)

Award ID: N/A (Pardoll)
Title: The Johns Hopkins University Bloomberg-Kimmel Institute for Cancer Immunology
Effort: 2.88 calendar months
Supporting agency: Bloomberg Philanthropies
Procuring Contracting/Grants Officer: Patricia Harris
Address of Grants Officer: 25 E. 78th St, New York, NY 10075
Performance period: 01/01/2016-12/31/2020
Level of funding:
Project's Goal(s): The goal of the Institute is to develop, within 10 years, immunotherapies that can place 50% of people with inoperable cancer into lifelong remission. The institute funds multiple immunotherapy related programs, cores, and pilot projects.
Specific Aims: N/A
Role: Institute Director
Overlap: None

Award ID: BMSC1259 (Pardoll)
Title: Strategic Research Collaboration Agreement
Effort: 2.88 calendar months
Supporting agency: Bristol Myers Squibb
Procuring Contracting/Grants Officer: Fouad Namoun
Address of Grants Officer: 345 Park Avenue, New York, New York 10154
Performance period: 01/01/2017-12/31/2022
Level of funding:
Project's Goal(s): This overarching contract funds multiple immunotherapy research projects and clinical trials in an effort to determine the IO agents or combination treatments incorporating IO agents that provide the optimal benefit risk ratio for cancer populations and subpopulations.(when applicable)
Specific Aims: N/A
Role: PI
Overlap: None

Award ID: CA209-596 (Lim)
Title: ABTC: A Phase I Trial of Anti-LAG-3 or Anti-CD137 Alone and in Combination with Anti-PD-1 in Patients with Recurrent GBM (ABTC 1501)
Effort: .02 calendar months
Supporting agency: Bristol Myers Squibb
Procuring Contracting/Grants Officer: Fouad Namoun
Address of Grants Officer: 345 Park Avenue, New York, New York 10154
Performance period: 04/29/2016-05/01/2020
Level of funding:
Project's Goal(s): The major goal of this project is assaying and analyzing the correlatives of CA209-596/ABTC1501 study.
Specific Aims: N/A

Role: Co-Investigator
Overlap: None

Award ID: W81XWH-17-1-0627 (Elisseeff)
Title: Removal of trauma induced senescent cells as a new treatment for osteoarthritis
Effort: .24 calendar months
Supporting agency: US Department of Defense
Procuring Contracting/Grants Officer: TBD
Address of Grants Officer: Unknown
Performance period: 09/30/2017-09/29/2020
Level of funding:
Project's Goal(s): The goal of this project is to answer the fundamental questions on how senescent cells induce OA after trauma (with implications also for age-related OA).
Specific Aims: 1.) Define the senescence and immunological profile of the articular joint after ACLT injury
2.) Determine the impact of senolytics and clearance of senescent cells on the immune profile and PTOA disease 3.) Develop and test controlled release senolytics in the PTOA murine model
Role: Co-Investigator
Overlap: None

AWARDED SINCE LAST SUBMISSION

Award ID: MFCR-MIC-001 (Pardoll)
Title: The MANAFEST Project
Effort: 1.2 calendar months
Supporting agency: The Mark Foundation for Cancer Research
Procuring Contracting/Grants Officer: Michele Cleary
Address of Grants Officer: 10 East 53rd St, Floor 13 New York, NY 10022
Performance period: 10/13/2017-10/12/2020
Level of funding:
Project's Goal(s): The major goal of this project is to leverage and extend the existing Genomics Data Commons (GDC) towards the development of a Cancer Immunology Relational Database that integrates immunotherapy clinical and immunologic information in a fashion that can be efficiently mined.
Specific Aims: 1.) Development of data standards for the newer immune related assays (such as neoantigen characterizations, immunophenotyping approaches. 2.) Map out a process for adapting the cBioPortal in which the features initially chosen have been shown to be important for modulating response to immunotherapies. 3.) Optimize, standardize and validate across institutions a new T cell assay platform – MANAFEST – together with informatics tools such as ImmunoMap, that will transform outputs into quantitative variables suitable for
populating the Cancer Immunology Relational Database.
Role: PI
Overlap: None

Award ID: (Anagnostou)
Title: Dynamics of neoantigen landscape during immunotherapy in lung cancer
Effort: .12 calendar months *effort, no salary support
Supporting agency: LUNGevity Foundation
Procuring Contracting/Grants Officer: Margery Jacobson
Address of Grants Officer: 228 S. Wabash Ave Suite 700, Chicago, IL 60604
Performance period: 11//01/2017-10/31/2020
Level of funding:

Project's Goal(s): The goal of this project is to us the results of the proposed research including identification of putative neoantigens identified prior to and at the time of emergence of resistance and development of liquid molecular assays predictive of resistance to immune checkpoint blockade will be used to develop patient-specific immunotherapy approaches in a variety of cancer types and will launch investigator-initiated clinical trials at Hopkins and other institutions.
Specific Aims: N/A
Role: Collaborator
Overlap: None

Award ID: N/A (Pardoll)
Title: Ervaxx-JHU Sponsored Research Agreement
Effort: .6 calendar months
Supporting agency: ERVAXX Limited
Procuring Contracting/Grants Officer: Timothy Edwards, Chief Operating Officer
Address of Grants Officer: 71, Kingsway, London WC2B 6ST, United Kingdom
Performance period: 12/20/2017-06/19/2020
Level of funding:
Project's Goal(s): The major goals of this project are to address the following questions: **1.)** Are there detectable CD8 and CD4 responses against certain HERV-related peptide antigens in cancer patients, **2.)** Which HERV-related peptides generate the best and most consistent(ie among multiple patients) responses, in particular relative to normal controls, **3)** Can HERV-related peptide-specific T cell clones be found in the tumors? **4.)** How do HERV-related peptide-specific responses compare with MANA-specific responses.
Specific Aims: see major goal above
Role: Collaborator
Overlap: None

COMPLETED SINCE LAST SUBMISSION

Award ID: N/A (Brahmer)
Title: Stand Up To Cancer-American Cancer Society Lung Cancer Translational Research Dream Team Grant: Targeting KRAS Mutant Lung Cancers
Effort: .6 calendar months
Supporting Agency: Mass General Hospital (AACR Prime)
Name of Procuring Contracting/Grants Officer: Ashley Gleason, Manager, Partners Research Management
Address of Grants Officer: 101 Huntington Ave, Suite 200 Boston, MA 02199
Period of Performance: 08/01/2015-07/31/2018
Level of Funding:
Project's Goal: As part of the Stand up to Cancer-American Society lung Cancer Translational Research Dream Team, efforts will focus on implementing advances in lung cancer research as rapidly as possibly through the creation of a collaborative, translational, cancer research "Dream Team**".**
Specific Aims: N/A
Role: Co-Investigator
Overlap: None

Award ID: N/A (Lim)
Title: Pre-Immunization During Delta24-RGD Therapy in Intracranial Gliomas
Effort: .24 calendar months
Supporting agency: DNATrix
Procuring Contracting/Grants Officer: Unknown

Address of Grants Officer: 2450 Holcombe Blvd Suite X+200 Houston, Texas 77021
Performance period: 08/02/2017-07/31/2018
Level of funding:
Project's Goal(s): See aims below
Specific Aims: 1.) To assess the optimal dosing strategy of Delta24-RGD therapy in GL261 gliomas (*pilot experiment*). 2.) To assess the anti-tumor effect of pre-immunization during Delta24-RGD therapy in GL261 gliomas 3.) To characterize immune cells involved in pre-immunized mice with GL261 gliomas during Delta24-RGD therapy
Role: Co-Investigator
Overlap: None

Award ID: N/A (Bollinger)
Title: miLab
Effort: .6 calendar months
Supporting agency: MiDiagnostics
Procuring Contracting/Grants Officer: 3001 Leuven, Kapeldreef 75, Belgium
Address of Grants Officer: Luc Van den Hove
Performance period: 06/01/2015-09/30/018
Level of funding:
Project's Goal(s): miLab is an *in-vitro* diagnostic system that integrates a complete clinical laboratory process including sample prep and bio-sensing capability on a single disposable chip-set. The platform will bring diagnostic tests to the point of need and to the point of care fundamentally changing the way we manage our health in the future.
Specific Aims: N/A
Role: Management Board Participant
Overlap: None

Individuals who have worked on the project

The Regents of the University of California

Name:	Dennis Slamon, M.D., Ph.D.
Project Role:	PI (Senior/Key Personnel)
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Slamon contributes clinical, translational, and genomic expertise to the project and is involved in the overall direction.
Funding Support:	See Other Support

Name:	Judy Dering, Ph.D.
Project Role:	Sr Public Analyst
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Dering is responsible for analyzing data from the microarray experiments.
Funding Support:	No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. See next pages for Dr. Slamon's Other Support.

OTHER SUPPORT

SLAMON, DENNIS

CURRENT

R01CA182514-01A1 (PI: Curtis)
Title: Intergrated genomic analysis and multi-scale modeling of therapeutic resistance
Time Commitment: 0.24
Supporting Agency: NIH Subaward with Stanford University
Procuring Contracting/Grants Officer: Aida Vasquez, Vasquez@mail.nih.gov
240-276-6319
Performance Period: 09/12/14-8/31/19
Level of Funding:
Project's Goal(s): The major goals of this project are to i) perform an integrated genomic analysis of serial tissue specimens from HER2-positive patients enrolled in clinical trials to evaluate the efficacy of single or dual agent neoadjuvant lapatinib and or trastuzumab targeted therapy (NCT00769470/TRIO B07) in order to characterize mechanisms of resistance ii) delineate temporal patterns of clonal expansions under treatment selective pressure by analyzing longitudinal samples collected prior to, at run-in, and after therapy iii) to functionally characterize mechanisms of resistance to single and dual agent therapy in HER2-positive tumors and to phenotype resistant cell populations by analyzing patient-derived xenograft models and short-term primary cultures.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

W81XWH-14-1-0385 (PI: Baylin)
Title: A New Paradigm for the treatment of Ovarian Cancer: The use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy.
Time Commitment: 0.60 Calendar Months
Supporting Agency: US Army Subaward with John Hopkins University
Procuring Contracting/Grants Officer: Melody Snow, M.H.S, Assistant Director, Outgoing Awards
Address of Grants Officer: John Hopkins University, School of Medicine, 1629 Thames Street, Suite 200
Baltimore, Maryland 21231
Performance Period: 9/30/2014-9/29/19
Level of Funding:
Project's Goal(s): To robustly prolong the survival of patients with serous ovarian cancer (OC) through introducing epigenetic therapy paradigms.
Specific Aims: 1) To uncover the mechanisms through which epigenetic therapy may, alone, achieve robust, durable responses in patients with advanced ovarian cancer (OC) 2) Study how epigenetic therapy may sensitize OC cells to subsequent chemotherapies 3) Study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance 4) Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC
5) Bring all of the above studies to bear on leveraging clinical trials of epigenetic therapy on OC
Project Overlap or Parallel: No scientific or budgetary overlap.

P30 CA016042 (PI: Teitell)
Title: "Cancer Support Grant"
Time Commitment: 2.0 calendar
Supporting Agency: NIH/NCI
Procuring Contracting/Grants Officer: Amy Connolly, Grant Management Specialist

Address of Grants Officer: National Cancer Institute, Room 700, Mail Stop 8335
6116 Executive Blvd, Bethesda, MD 20852-8335
Performance Period: 4/23/2003-11/30/2018
Level of Funding:
Project's Goal(s): This Funding supports activities to increase scientific interaction among members of the Signal Transduction Program Area at Jonsson Cancer Center.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

CIRM DR3-07067 (PI: Slamon)
Title: "A Phase I dose escalation and expansion clinical trial of the novel first-in-class Polo-like Kinase 4 (PLK4) inhibitor, CFI-400945 in patients with advanced solid tumors"
Time Commitment: 3.60 calendar
Supporting Agency: California Institute for Regenerative Medicine
Procuring Contracting/Grants Officer: Doug Kearney, Grants Management Office
Address of Grants Officer: California Institute for Regenerative Medicine, 210 King Street
San Francisco, CA 94107
Performance Period: 05/01/2014-10/31/2018 (NCE)
Level of Funding:
Project's Goal(s): This proposal is aimed at a phase I clinical trial of CFI-400945, a first-in-class inhibitor of Polo-like Kinase 4 (PLK4). PLK4, a serine/threonine kinase functions at the intersection of mitosis, DNA repair, hypoxia and metabolism, and is expressed in a variety of solid tumors. Overexpression of PLK4 results in the excessive formation of centrioles and multinucleation in cells suggesting that the elevated expression of PLK4 in tumors could contribute to chromosomal instability (CIN) and aneuploidy. Of interest, PLK4 overexpression in neural stem cells drives centrosome amplification and is associated with tumor formation. Conversely, depletion of PLK4 in cancer cells by RNA interference prevents centriole duplication, causing mitotic defects and cell death. Notably, these effects are amplified in hypoxic conditions. Thus, PLK4 is an attractive target for the development of small-molecule therapeutics in cancer. The candidate molecule, CFI-400945 was developed as part of a collaborative effort funded by CIRM/CSCC (PIs: Dennis Slamon and Tak Mak) that supported a drug discovery effort, preclinical assessment, and IND enabling studies.
Specific Aims: This clinical trial described herein will be carried out in two parts. Part A will consist of the dose escalation phase of the first-in-human trial, where the primary objective will be to determine the maximum tolerated dose (MTD) of CFI-400945. In Part A, patients with any solid tumor refractory to conventional treatment will be enrolled in order to reach the MTD expeditiously. Part B will consist of the expansion phase, where the primary objectives are to further refine the MTD to assist in determination of the recommended phase II dose (RP2D), to further assess plasma pharmacokinetics and to evaluate preliminary evidence of antitumor activity patient populations dosed at the MTD. Up to 4 expansion cohorts of 6-12 patients each would be enrolled which may include: 1) cohorts restricted to a specific tumor histology and/or specific biomarker (predicated upon preclinical data) and a 2) a biomarker cohort to obtain tumor biopsy samples at pre-treatment, on-treatment, with the exploratory objective of evaluating pharmacodynamic effects and potential resistance mechanisms. We expect that the dose escalation will complete enrollment in approximately 1 year and an additional 12-18 months for completion of the expansion cohorts. We then expect an additional one year period will be required to collect data and complete a clinical study report (CSR). We believe that this Phase 1/1B trial will provide critical clinical and biomarker data that will demonstrate clinical proof of concept which will inform the Phase 2 development plan. Over the next 4 years, our Phase I trial will also advance a successfully completed CIRM funded-project for which an IND has already been filed
Project Overlap or Parallel: No scientific or budgetary overlap.

SNDX-275-0602 (PI: Slamon)
Title: A Randomized, Placebo-Controlled, Double-Blind, Multicenter Phase 2 Study of Atezolizumab With or Without Entinostat in Patients with Metastatic Triple Negative Breast Cancer, with a Phase Ib Lead in Phase (SYNDX-275-0602)
Time Commitment: 0.12 calendar
Supporting Agency: Syndax
Procuring Contracting/Grants Officer: Launa Aspeslet
Address of Grants Officer: 9925 109th St NW, Suite 1100; Edmonton T5K2J8, Alberta, Canada.
Performance Period: 4/28/2016-04/28/2019
Level of Funding:
Project's Goal(s): To determine the dose-limiting toxicities (DLT) and maximum tolerated dose (MTD) or recommended Phase 2 dose (RP20) or entinostate (SNDX-275) giving in combination with atezolizumab.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

CLEE0111F2301 (PI: Slamon)
Title: A randomized double-blind, placebo-controlled study of ribociclib in combination with fulvestrant for the treatment of postmenopausal women in hormone receptor positive, HER2-negative, advanced breast cancer who have received no or only one line of prior endocrine treatment.
Time Commitment: 0.12 calendar
Supporting Agency: Novartis
Procuring Contracting/Grants Officer: Karen Riccardello
Address of Grants Officer: One Health Plaza, East Hanover, NJ 07936-1080
Performance Period: 04/20/2015-05/08/2021
Level of Funding:
Project's Goal(s): To determine whether treatment with fulvestrant + ribociclib prolongs PFS compared to treatment with fulvestrant + ribociclib placebo in postmenopausal women with HR+, HER2- advanced breast cancer who received no or only 1 line of prior hormonal therapy for advanced breast cancer.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

CLEE0111H2301 (PI: Slamon)
Title: A phase III, multicenter, randomized, double-blind, placebo controlled study to evaluate efficacy and safety of ribociclib with endocrine therapy as an adjuvant treatment in patients with hormone receptor-positive, HER2-negative, intermediate risk early breast cancer.
Time Commitment: 0.12 calendar
Supporting Agency: Novartis
Procuring Contracting/Grants Officer: Karen Riccardello
Address of Grants Officer: One Health Plaza, East Hanover, NJ 07936-1080
Performance Period: 04/20/2015-05/08/2021
Level of Funding:
Project's Goal(s): To compare invasive disease-free survival (iDFS) for ribociclib + ET versus placebo + ET in patients with HR positive, HER2-negative, EBC with intermediate risk of recurrence.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

C31003/TRIO-027 (PI: Slamon)
Title: An Open-Label Phase 2 Study of MLN0128 (A TORC1/2 Inhibitor) in Combination With Fulvestrant in Women with ER-Positive/HER2-Negative Advanced or Metastatic Breast Cancer That Has Progressed During or After Aromatase Inhibitor Therapy.
Time Commitment: 0.12 calendar
Supporting Agency: Millennium
Procuring Contracting/Grants Officer: Maria Alanlzas
Address of Grants Officer: 40 Landsdowne Street, Cambridge, MA 02139. USA
Performance Period: 10/26/2016-10/26/2019
Level of Funding:
Project's Goal(s): To compare the PFS of patients treated with the combination of fulvestrant+daily MLN0128 versus patients treated with single-agent fulvestratn. To compare the PFS of patients treated with the combination of fulvestrant+weekly MLN0128 versus patients treated with single-agent fulvestrant.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

TRIO-030 (PI: Slamon)
Title: A Presurgical Tissue-Acquisition Study to Evaluate Molecular Alterations in Human Breast Cancer Tissue Following Short-Term Exposure to the Androgen Receptor Antagonist ODM-201
Time Commitment: 0.12 calendar
Supporting Agency: TRIO
Procuring Contracting/Grants Officer: Launa Aspeslet
Address of Grants Officer: 9925 109th St NW, Suite 1100; Edmonton T5K2J8, Alberta, Canada.
Performance Period: 03/29/2017-03/29/2020
Level of Funding:
Project's Goal(s): To identify the molecular alterations that occur in hum BC tissue, following short-term exposure to ODM-201 in female subjects with EBC. To evaluate the safety and tolerability of short-term exposure to ODM-201 in female subjects with EBC.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

AWARDED SINCE LAST SUBMISSION

None

COMPLETED SINCE LAST SUBMISSION

A5481023 (PI: Slamon)
Title: Multicenter, Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial of Fulvestrant (Faslodex®) With or Without PD-0332991 (Palbociclib) ± Goserelin in Women with Hormone Receptor-Positive, HER2-Negative Metastatic Breast Cancer Whose Disease Progressed After Prior Endocrine Therapy.
Time Commitment: 0.12
Supporting Agency: Pfizer
Procuring Contracting/Grants Officer: Soo Y. Bang
Address of Grants Officer: Address of Contract officer: 235 E. 42nd Street, MS 685/13/1, New York, New York 10017
Performance Period: 11/26/13-11/26/17
Level of Funding:
Project's Goal(s): is to demonstrate the superiority of palbociclib in combination with fulvestrant (with or without goserelin) over fulvestrant alone (with or without goserelin) in prolonging investigator-assessed PFS in women with HR+/HER2-negative metastatic breast

cancer whose disease has progressed on prior endocrine therapy.
Specific Aims: N/A
Project Overlap or Parallel: No scientific or budgetary overlap.

OAM4861g (PI: Slamon)
Title: A randomized, phase ii, multicenter, double-blind, placebo-controlled study evaluating the safety and efficacy of metmab and/or bevacizumab in combination with paclitaxel in patients with metastatic triple-negative breast cancer.
Time Commitment: 0.12 calendar
Support Agency: Genentech Inc.
Procuring Contact: Michael Smith (Associate Director) Michaelj.smith@syneoshealth.com
Performance Period: 12/01/2011 – 02/28/2015
Level of Funding:
Project's goals: To estimate the clinical benefit of onartuzumab + bevacizumab + paclitaxel and onartuzumab + placebo + paclitaxel relative to placebo + bevacizumab + paclitaxel, as measured by investigator-assessed progression-free survival (PFS), in patients with metastatic or locally recurrent, triple-negative breast cancer who have received no prior systemic therapy or have progressed following first-line therapy for metastatic disease.

Individuals who have worked on the project

Van Andel Research Institute

Name:	Peter Jones, Ph.D.
Project Role:	PI (Senior/Key Personnel)
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Jones serves as PI on this project.
Funding Support:	See Other Support

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. See next pages for Dr. Jones' Other Support.

OTHER SUPPORT

JONES, PETER A.

CURRENT

W81XWH14-1-0385 (PI: Baylin)

Title:	A New Paradigm for the Treatment of Ovarian Cancer: The Use of Epigenetic Therapy to Sensitize Patients to Immunotherapy and Chemotherapy
Time Commitment:	0.36 calendar
Supporting Agency:	DoD/Department of the Army via Johns Hopkins University
Procuring/Contracting/Grants Officer:	Barbara Schneider, Johns Hopkins University,
Address of Grants Officer:	The Sidney Kimmel Comp Cancer Ctr, 1650 Orleans St., CRBI Rm352, Baltimore, MD, 21287-0013, schneba@jhmi.edu
Performance Period:	9/30/14 – 9/30/19
Level of Funding:	
Project Goals:	The goal of this project is to determine how DNMTs activate drug response pathways in ovarian cancer.
Specific Aims:	1) To uncover the mechanisms through which epigenetic therapy may, alone, achieve robust, durable responses in patients with advanced ovarian cancer (OC); 2) Study how epigenetic therapy may sensitize OC cells to subsequent chemotherapies; 3) Study how epigenetic therapy may sensitize OC cells to subsequent immunotherapies which targets checkpoints which are driving immune tolerance; 4) Develop new combinations of epigenetic drugs which may provide for the highest level of efficacy for management of OC; 5) Bring all of the above studies to bear on leveraging clinical trials of epigenetic therapy on OC
Project Overlap or Parallel:	No scientific or budgetary overlap.

R35 CA209859 (PI: Jones)

Title:	Targeting DNA Methylation and the Cancer Epigenome
Time Commitment:	6.0 calendar
Supporting Agency:	NIH/NCI
Procuring Contracting/Grants Officer:	Long Nguyen
Address of Grants Officer:	National Cancer Institute, Executive Plaza North, Suite 5024, 6130 Executive Blvd., Rockville, MD 20852
Performance Period:	01/01/17-12/31/23
Level of Funding:	
Project's Goals:	This major goal of this project is to provide new approaches to treating cancers through the many epigenetic changes seen in the genomes of cancer cells.
Specific Aims:	Future Question 1: Why are there so many mutations in chromatin modifiers and what are the effects of these mutations on the structure of the epigenome? Future Question 2: What are the functional consequences of activating the expression of cancer/testis genes by 5-Aza-CdR? Future Question 3: What double-stranded RNAs are activated by 5-Aza-CdR and how do these relate to cellular responses? Future Question 4: Can we

	design combinations of epigenetic drugs which might increase the effectiveness of 5-azanucleoside treatment? Future Question 5: Can cryo-EM help to visualize complexes relevant to chromatin structure and functions?
Project Overlap or Parallel:	No scientific or budgetary overlap.

AWARDED SINCE LAST SUBMISSION

SU2C-AACR-CT01-16 (PI: Baylin and Hellman)

Title:	Combined Epigenetic Therapy and Pembrolizumab for Advanced NSCLC
Time Commitment:	0.3 calendar
Supporting Agency:	AACR
Procuring Contracting/Grants Officer:	Unknown
Address of Grants Officer:	Unknown
Performance Period:	04/01/17-03/31/20
Level of Funding:	
Project's Goals:	The primary objective of this project is to test a combined epigenetic therapy to determine whether it will result in a better immune response to cancer and improve the proportion of patients who respond.
Specific Aims:	N/A
Project Overlap or Parallel:	No scientific or budgetary overlap

SU2C-AACR-CT08-17 (PI: Jones and Plimack)

Title:	Overcoming Urothelial Cancer Atezolizumab Resistance by Epigenetic Therapy
Time Commitment:	0.6 calendar
Supporting Agency:	AACR
Procuring Contracting/Grants Officer:	Unknown
Address of Grants Officer:	Unknown
Performance Period:	11/01/17-10/31/20
Level of Funding:	
Project's Goals:	The major goal of this project is a clinical trial to evaluate the ability to resensitize relapsed/ refractory bladder cancer patients who have failed immunotherapy.
Specific Aims:	N/A
Project Overlap or Parallel:	No scientific or budgetary overlap

COMPLETED SINCE LAST SUBMISSION

Not applicable

8. SPECIAL REPORTING REQUIREMENTS

Progress of the Teal Junior Scientists

Scientific training – The progress of all our TEAL trainees has been well outlined above and key points are re-summarized here.

As mentioned in the progress report, Dr. Chiappinelli benefitted enormously from her time with us. For her studies in the Cell, 2015 paper, she worked with collaborators in Germany, Drs. Reiner Strick and Pamela Strissel for learning how to profile, knockdown, and overexpress several of the endogenous retroviruses (ERVs). She was also mentored extensively, with Dr. Cindy Zahnow's group for the mouse model work, the work for which is a co-author on Meredith's Stone's PNAS paper in 2017. Kate has now been a faculty member at George Washington University in the Cancer Center for two years or so and she is thriving from all interactions we have had with her. She continues some collaborative work with us and particular with our current trainee, Dr. Topper and all is concentrated on ovarian cancer research.

Scientific training – Dr. Meredith Stone: For her year as a mentee Meredith Stone worked with Dr. Cindy Zahnow for her dissertation studies and was co-mentored by Dr. Baylin. She benefitted from all of the activities listed above for Dr. Chiappinelli and, as fully outlined in the progress report, her work resulted in her paper now in press in PNAS on the mouse OC model. All of the educational activities and mentoring activities outlined for Dr. Chiappinelli were engaged in by Dr. Stone. As reported, Dr. Stone has now been a postdoctoral fellow at University of Pennsylvania in the Cancer Center where she has continued for a year now working on aspects of immunology related to OC.

Scientific training – Dr. Michael Topper: As outlined in the Progress Report, over the past year our newest mentee, Dr. Topper is a postdoctoral fellow with Dr. Baylin. In studies since his first author Cell paper in 2017, and his invaluable collaboration with Drs. Stone and Zahnow on their 2017 PNAS paper, his work remains simply outstanding. Over this next year, he is working to extend the implications of his epigenetic therapy development for OC. He is also concentrating deeply with Dr. Chiappinelli on a deep molecular analysis of multiple OC cell lines treated with the epigenetic therapy paradigms he has developed. The goal of these studies is to outline in novel ways, the upstream mechanisms by which the epigenetic therapy helps reverse tumor evasion including defining upstream signals that drive the entire induced viral defense response.

Presentations for the above trainees:

Participation in Hopkins groups: All three mentees participated in the following:

1) Methylation Data Group: attended these weekly meetings, and presented several times per year.
2) Methylation Journal Club: attended these weekly meetings, and presented many times.
3) Tumor Biology Lab Meeting: attended these weekly meetings, and presented at least twice per year.
4) Dr. Topper continues his collaborative work with co-investigator Peter Jones and his group at the Van Andel Research Institute in Grand Rapids, Michigan.

Professional development:

As above all three mentees had great development of their professional careers directly dependent on their training time as completely outlined just above.

Additional training: As above.

Individual Development Plan (IDP) for Postdoctoral Fellows:
Johns Hopkins University School of Medicine requires postdoctoral fellows and their mentors to fill out an annual IDP. This allows the fellow and mentor to identify long-term and short-term goals for the postdoc's research progress as well as career development. All three mentees have completed IDP's and this has been reviewed annually with Drs. Baylin and Zahnow.

Teal Innovator's Ovarian Cancer ambassadorship activities

More this past year than ever, Dr. Baylin has been requested to discuss, nationally and internationally the exciting results which are outlined in the progress report and which constitute the 2017 Cell and PNAS papers. This past year, he has lectured at many places (see Presentations on pgs. 9-10) and is due to give a major lecture to the School of Medicine at Vanderbilt University in November of this year.

9. APPENDICES

Liu M, Thomas SL, DeWitt AK, Zhou W, Madaj ZB, Ohtani H, Baylin SB, Liang G and Jones PA. Dual inhibition of DNA and histone methyltransferases increases viral mimicry in ovarian cancer cells. Cancer Research 78(20): 5754-5766, 2018.

Published OnlineFirst September 5, 2018; DOI: 10.1158/0008-5472.CAN-17-3953

Genome and Epigenome

Cancer Research

Dual Inhibition of DNA and Histone Methyltransferases Increases Viral Mimicry in Ovarian Cancer Cells

Minmin Liu[1], Stacey L. Thomas[1], Ashley K. DeWitt[1], Wanding Zhou[1], Zachary B. Madaj[1], Hitoshi Ohtani[1], Stephen B. Baylin[1,2], Gangning Liang[3], and Peter A. Jones[1]

Abstract

Ovarian cancer ranks as the most deadly gynecologic cancer, and there is an urgent need to develop more effective therapies. Previous studies have shown that G9A, a histone methyltransferase that catalyzes mono- and dimethylation of histone H3 lysine9, is highly expressed in ovarian cancer tumors, and its overexpression is associated with poor prognosis. Here we report that pharmacologic inhibition of G9A in ovarian cancer cell lines with high levels of *G9A* expression induces synergistic antitumor effects when combined with the DNA methylation inhibitor (DNMTi) 5-aza-2′-deoxycytidine (5-aza-CdR). These antitumor effects included upregulation of endogenous retroviruses (ERV), activation of the viral defense response, and induction of cell death, which have been termed "viral mimicry" effects induced by DNMTi. G9Ai treatment further reduced H3K9me2 levels within the long terminal repeat regions of ERV, resulting in further increases of ERV expression and enhancing "viral mimicry" effects. In contrast, G9Ai and 5-aza-CdR were not synergistic in cell lines with low basal *G9A* levels. Taken together, our results suggest that the synergistic effects of combination treatment with DNMTi and G9Ai may serve as a novel therapeutic strategy for patients with ovarian cancer with high levels of G9A expression.

Significance: Dual inhibition of DNA methylation and histone H3 lysine 9 dimethylation by 5-aza-CdR and G9Ai results in synergistic upregulation of ERV and induces an antiviral response, serving as a basis for exploring this novel combination treatment in patients with ovarian cancer. *Cancer Res; 78(20); 5754–66. ©2018 AACR.*

Introduction

Recent advances in cancer genomics and epigenomics have revealed that the vast majority of human cancers harbor both genetic and epigenetic alterations (1–3). Epigenetic mechanisms, including DNA and histone modifications, as well as chromatin accessibility, determine how cells express genes and play a crucial role in regulating normal cellular functions (4). Aberrant DNA methylation occurs in almost every cancer type and is among the earliest and most common event during tumorigenesis (5). Unlike genetic abnormalities, which are difficult to reverse, epigenetic alterations are readily reversible, making them attractive therapeutic targets for cancer treatment (6).

A group of inhibitors that target epigenetic modifiers has emerged as an exciting group of compounds for use in the clinic (6). For example, the FDA has approved DNA methyltransferase inhibitors (DNMTi), such as 5-aza-2′-deoxycytidine (5-aza-CdR), for the treatment of myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Currently, there are ongoing clinical trials for solid tumors including colon, ovarian, liver, lung, and breast cancer (6, 7). Proposed mechanisms underlying the clinical efficacies of DNMTis include demethylation of the promoters of tumor suppressor genes and bodies of oncogenes, thereby restoring more normal expression levels of these genes (8). Moreover, recent work has suggested a novel mechanism of action for DNMTis, termed "viral mimicry" (9–11). Viral mimicry is characterized by the upregulation of endogenous retrovirus (ERV) transcripts, formation of cytoplasmic double-stranded RNA, and the induction of viral defense pathways (9–11). Importantly, the sensing of these ERVs by viral defense proteins leads to the death of colon cancer stem cells (11). The response to DNMTis in a subset of patients was impressive, with long-term durable antitumor effects (12–15).

However, primary and secondary resistance to the epigenetic therapies is common, likely because epigenetic processes are established and reinforced by both positive and negative feedback loops (16, 17). For example, while DNMTis induce immediate and genome-wide demethylation of DNA, their effectiveness can be limited by a fairly rapid remethylation after the drug is removed (8), and/or by the involvement of alternative silencing mechanisms such as histone modifications (18). The future of epigenetic therapies, especially for solid tumors, relies on the rational design of combination treatments taking alternative silencing mechanisms into consideration.

DNA methylation plays an important role in silencing ERVs in somatic cells and in the male differentiating germline (19–22), yet

[1]Van Andel Research Institute, Grand Rapids, Michigan. [2]Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland. [3]Department of Urology, Keck School of Medicine, University of Southern California, Los Angeles, California.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

M. Liu, S.L. Thomas, and A.K. DeWitt contributed equally to this article.

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it is dispensable for the repression of a subset of ERVs in the early germline, early embryo, and embryonic stem (ES) cells (23–25). Silencing of ERVs in early embryo and germline development depends primarily on histone methylation, notably at lysine 9 of histone H3 (H3K9). Many studies have shown that H3K9 lysine methyltransferases, including G9A, GLP, and SETDB1, are essential for repressing ERV expression in these stages (24–27). Importantly, G9A, which is responsible for catalyzing mono- or dimethylation of H3K9, is upregulated in many types of cancers (28–30). G9A and H3K9me2 are present at DNA hypermethylated promoters of tumor suppressor genes in cancers and are lost from the promoters when these genes are reactivated by DNMTis or in DKO cells (31, 32). The level of *G9A* overexpression has been associated with poor prognosis of ovarian, colon, breast, and lung cancers (30, 33–36). We have previously shown that knocking down G9A sensitizes a colon cancer cell line to 5-aza-CdR treatment (37). In addition, G9Ai in combination with DNMTis has been proposed as a potential therapy for sickle cell disease, because this combination further activates fetal hemoglobin genes (38). This combination has also been shown to effectively increase the expression of immune genes in a colon cell line (39). These findings urged us to evaluate this novel combination as a potential epigenetic therapy approach for ovarian cancer. Here, we show that pharmacologic inhibition of G9A synergistically enhances the efficacy of 5-aza-CdR by restoring strong promoter activity of ERVs and enhancing the viral mimicry effects in ovarian cancer cells with high levels of *G9A* and *GLP* expression.

Materials and Methods

Cell lines and drugs

A2780, CAOV3, PEO14, and OAW42 ovarian cancer cell lines were authenticated using the ATCC human cell line authentication service. All cell lines were cultured according to standard mammalian tissue culture protocols using sterile technique. A2780 were maintained in McCoy's 5A medium, PEO14 cells in RPMI 1640 medium and CAOV3 and OAW42 in Dulbecco's Modified Eagle Medium. All media (from GIBCO) were supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 1% penicillin/streptomycin (GIBCO). Venor GeM Mycoplasma Detection Kit (Sigma-Aldrich) was used every 3 months to confirm all cell lines were *Mycoplasma*-free. 5-Aza-CdR and G9Ai (UNC0638) were purchased from Sigma-Aldrich and APExBIO, respectively.

Cell viability assay

To test for dose-dependent response, 400 A2780 cells or 1,000 CAOV3, PEO14, and OAW42 cells were plated in each well of the 96-well plates 24 hours prior to the treatment. A2780 and OAW42 cells were exposed to a dose of 5-aza-CdR (from 25 to 4,800 nmol/L) for 48 hours, while CAOV3 and PEO14 cells were treated with 2 consecutive daily doses of 5-aza-CdR (from 25 to 4,800 nmol/L) for 48 hours. Subsequently G9Ai UNC0638 (from 100 to 20,000 nmol/L) was added to the culture media until 7 days after the treatment. Cells were then incubated with CellTiter-Glo assay reagent (Promega) for 10 minutes and luminescence was measured using a Synergy HT multimode microplate reader (BioTek).

Evaluation of combination effect

Cell viability data were normalized to their corresponding untreated controls for each treatment condition and were expressed as percentage fractional affect (Fa). CompuSyn software (ComboSyn, Inc.) was used to calculate combination index (CI) values of Fa under different conditions using the Chou–Talalay equation (40) $CI = (D)_{Vc}/((D_m)_{Vc}\ (Fa/(1\text{-}Fa))^{1/m1}) + (D)_{Aza}/((D_m)_{Aza}\ (Fa/(1\text{-}Fa))^{1/m2})$, where D is the concentration of G9Ai and 5-aza-CdR either alone or in combination to achieve a given Fa. The median-effect dose (D_m), $m1$ (G9Ai), and $m2$ (5-aza-CdR) values were determined using the median-effect equation (41) $(Fa)/(1\text{-}Fa) = ((D)/(D_m))^m$ for G9Ai and 5-aza-CdR treatment alone. The CI values define synergistic effect (42) when $CI < 1$, additive effect when $CI = 1$, and antagonism when $CI > 1$.

Cell-cycle analysis and quantification of dead cell percentages

Cells were harvested and then stained with the amine reactive viability dye Ghost Dye Violet 450 (Tonbo Biosciences) at 4°C for 30 minutes according to the manufacturer's protocol. Cells were then fixed with 66% ethanol and stored at 4°C until ready to stain with propidium iodide (PI). Fixed cells were pelleted and resuspended in 500 μL of PI staining solution (PBS + 100 μg/mL RNase A + 50 μg/mL PI) and incubated overnight at 4°C. For flow cytometry analysis, cells were filtered through cell strainer snap caps (Fisher Scientific) and then analyzed on a CytoFLEX S (Beckman Coulter). Cell cycle was analyzed using ModFit LT software (Verify Software House, www.vsh.com), and the percentage of cells alive and dead was measured using FlowJo v10.0.7 (FlowJo, LLC).

Chromatin fractionation and Western blot analysis

Cell lysis and washing steps were performed in cold buffer containing 10 mmol/L PIPES, pH 7.0, 300 mmol/L sucrose, 100 mmol/L NaCl, 3 mmol/L MgCl2, 1 × EDTA-free protease inhibitor (Roche), 1 × phosphatase inhibitor cocktail (Sigma), and 0.1% Triton X-100. Whole-cell and chromatin fractions were treated with benzonase (Sigma-Aldrich) prior to Western blot analysis. Whole-cell, chromatin-associated or soluble fractions were mixed with SDS/β-mercaptoethanol loading buffer and resolved on a Bio-Rad 4% to 15% gradient SDS/PAGE gel. Antibodies against G9A (Perseus Proteomics Inc., PP-A8620A-00), Tubulin (Cell Signaling, 86298S), TATA-binding protein (TBP; Santa Cruz Biotech sc-74596), H3K9me1 (Epicypher 13-0014), H3K9me2 (Abcam #1220), H3K9me3 (Active Motif #39161), and total H3 (Abcam #12079) were used. Proteins were visualized using the Clarity Western ECL substrate (Bio-Rad) and ChemiDoc XRS+ imaging system (Bio-Rad).

RNA-seq

Total RNA was extracted with TRIzol reagent (Invitrogen), followed by cleanup and Turbo DNase I (Invitrogen) treatment with a Zymo Direct-Zol RNA mini prep kit (Zymo Research) according to the manufacturer's protocol. RNA quality was assessed using Agilent 2100 bioanalyzer with RNA Nano chips (Agilent Technologies, Inc.). For directional RNA-seq with ribosomal RNA (rRNA) reduction, libraries were prepared using the KAPA Stranded RNA-seq Kit with RiboErase (HMR; KapaBiosystems) and sequenced as single-end 75 bases on a NextSeq 500 instrument (Illumina) at the Van Andel Research Institute Genomics Core. The RNA-seq reads were mapped to the human transcriptome using TopHat version 2.1.0 with NCBI RefSeq as the reference annotation of transcripts. The transcripts were assembled and quantified using Cufflinks version 2.2.1.

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Differential expression is measured using edgeR package from the Bioconductor project.

Identification of bidirectionally transcribed ERVs

To quantify the transcription of repetitive element at a specific locus, we used Repeatmasker (http://www.repeatmasker.org) annotation as our input and considered only uniquely mapped reads (with mapping quality threshold 10) as previously described (10). For each transcript, we separated reads mapped to the two strands. We considered a transcript as bidirectionally transcribed if the smaller read count divided by the greater read count is over 0.5.

MethyLight assay and bisulfite sequencing

On day 5 after treatment, A2780, CAOV3, PEO14, and OAW42 cells were harvested, and genomic DNA was purified by phenol–chloroform extraction and ethanol precipitation. Bisulfite conversion was performed using the EZ DNA Methylation Kit (Zymo). MethyLight assays were then performed as previously described (43) using the primers listed in Supplementary Table S1. For A2780 cells, bisulfite PCR was performed using bisulfite-converted DNA with primers listed in Supplementary Table S1, and the products were cloned using the pGEM-T Vector System I (Promega) as previously described (10).

Chromatin immunoprecipitation

Single nucleosome preparation was performed according to the Dilworth lab native chromatin immunoprecipitation (ChIP) protocol (44). Briefly, A2780 cells (10^7 cells) before and after 5-aza-CdR, G9Ai, or combination treatment were harvested and washed twice and resuspended in ice-cold buffer N (15 mmol/L Tris pH 7.5, 15 mmol/L NaCl, 60 mmol/L KCl, 8.5%(w/v) sucrose, 5 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 1 mmol/L DTT, 200 μmol/L PMSF, 1× cOmplete Mini EDTA-free Protease Inhibitor Cocktail; Roche). To prepare nuclei, cells were lysed in 1 mL Lysis Buffer (Buffer N supplemented with 0.3% NP-40 substitute; Sigma) for 10 minutes at 4°C, and nuclei were collected by centrifugation (500 × *g* for 5 minutes at 4°C), resuspended in 1 mL of Buffer N, then sedimented through 7.5 mL sucrose cushion (10 mmol/L HEPES pH7.9, 30%(w/v) sucrose, 1.5 mmol/L $MgCl_2$ and centrifuged 13,000 × *g* using Sorvall swinging bucket for 12 minutes at 4°C). To isolate single nucleosomes, the nuclei were digested with MNase (1U Worthington MNase per 70 μg of chromatin at 37°C for 10 minutes), the nucleosomes were then purified by hydroxyapatite chromatography, and adjusted to a concentration of 20 μg/mL with ChIP Buffer 1(25 mmol/L Tris pH 7.5, 5 mmol/L MgCl2, 100 mmol/L KCl, 10% (v/v) glycerol, 0.1% (v/v) NP-40 substitute) and analyzed using 2% agarose gel.

H3K9me2, H3K4me3, and H3K27ac ChIP were performed as previously described (45), using 5 μg of nucleosome pulled down with 10 μg of anti-H3K9me2 (Abcam ab1220), anti-H3K4me3 (Abcam ab1012), and anti-H3K27ac (Active Motif AM39133) antibody on Dynabeads Protein G (Invitrogen) for 2 hours at 4°C. Initial chromatin (10%) for each immunoprecipitation was set aside to serve as ChIP input. The beads were washed 3 times with ChIP Buffer 2 (10 mmol/L Tris pH 7.5, 5 mmol/L MgCl2, 300 mmol/L KCl, 10% (v/v) glycerol, 0.1% (v/v) NP-40 substitute), twice with ChIP Buffer 3 (10 mmol/L Tris pH 7.5, 250 mmol/L LiCl, 1 mmol/L EDTA, 0.5% Na • deoxycholate, 0.5%(v/v) NP 40 substitute), twice with 1 × TE buffer followed with two elution steps in elution buffer (50 mmol/L Tris pH 7.5, 1 mmol/L EDTA, 1% w/v SDS). After proteinase K (Roche) digestion (65°C 1 hour), sample DNA was purified using Agencourt AMPure XP beads (Beckman Coulter) prior to qPCR analysis. Primers used in this study are listed in Supplementary Table S1.

Accession codes

All data have been deposited at the Gene-Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) with accession code GSE108223.

Results

Combination treatment with G9Ai and 5-aza-CdR induces synergistic effects to promote cell death in ovarian cancer lines A2780 and CAOV3, but not in PEO14 and OAW42

RNA-seq data from The Cancer Genome Atlas (TCGA) project reveal that *G9A* expression is upregulated in most types of cancer, and that the highest levels are found in primary ovarian tumors among 33 cancer types (Supplementary Fig. S1A). In addition, the related histone methyltransferase *GLP* is also significantly overexpressed in ovarian tumors compared with normal tissues (Supplementary Fig. S1B). We therefore used four ovarian cancer cell lines A2780, CAOV3, PEO14, and OAW42 as our *in vitro* models for treatment. We first tested various concentrations of a G9A inhibitor (UNC0638) in A2780 cells and found that the global levels of H3K9me1 and H3K9me2 were efficiently reduced by 400 nmol/L G9Ai at 48 hours after the treatment (Fig. 1A). In addition, UNC0638 at 400 nmol/L exhibited low cellular toxicity for all four ovarian cancer cell lines (Supplementary Fig. S2). This is consistent with a previous report that UNC0638 has high G9A/GLP inhibition potency and low cellular toxicity with concentrations in the nanomolar range (46). We therefore used 400 nmol/L UNC0638 to determine the optimum dosing schedules for combination treatments with low-dose 5-aza-CdR (100 nmol/L) in A2780 cells. We explored different time points to add G9Ai relative to 5-aza-CdR and varied the duration of exposure to these compounds (Fig. 1B). The expression levels of *HERV-Fc1*, a marker for the effects of DNMTis (9, 10), were used as readout and were measured by quantitative RT-PCR. The dosing schedule in which A2780 cells were exposed to 5-aza-CdR for 48 hours and subsequently G9Ai until harvest achieved the greatest effect on upregulating *HERV-Fc1* (Fig. 1B). Therefore, this schedule was applied to A2780 and OAW42 cells in subsequent experiments (Fig. 2A). Because the doubling times for CAOV3 and PEO14 cells were greater than 24 hours, we treated these cells with two consecutive daily doses of 5-aza-CdR for 48 hours to compensate for the fact that incorporation of the drug requires cell doubling (Fig. 2B).

Following these dosing schedules, we tested whether the combinations of G9Ai and 5-aza-CdR could induce synergistic effects in ovarian cancer cells. We measured dose-dependent inhibition of cell proliferation [as fraction affected (f_a)] with various concentrations of 5-aza-CdR and G9Ai alone or in combination using the CellTiter-Glo luminescent cell viability assay (Supplementary Fig. S3). Combination treatments resulted in increased inhibition of cell proliferation (increased f_a) compared with single-compound treatment in A2780 and CAOV3 cells (Supplementary Fig. S3). The Chou–Talalay analyses (42) determined that the two compounds indeed acted synergistically (CI values <1) under the majority of conditions in A2780 and CAOV3 cells (Fig. 2C). In

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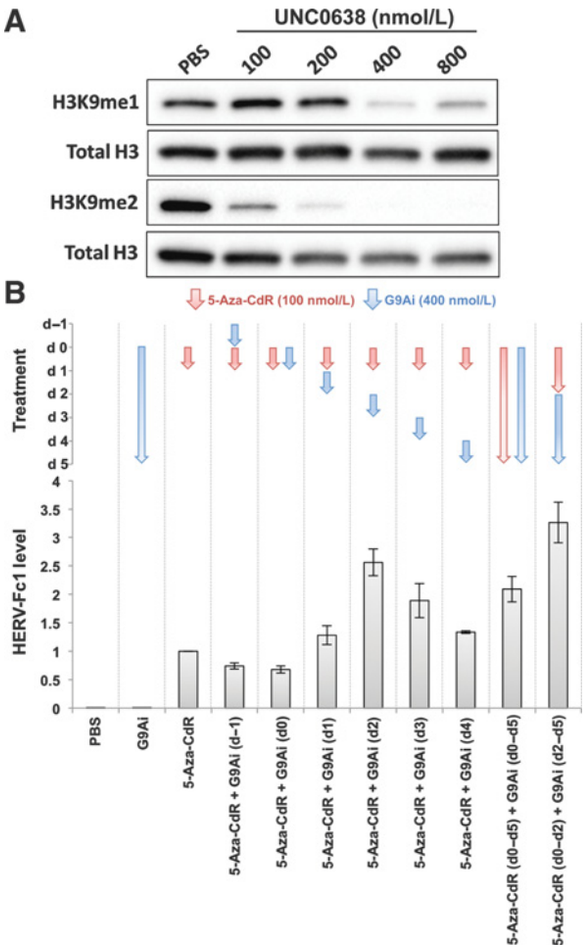

Figure 1.

Optimization of treatment schedule by combination of G9Ai and 5-aza-CdR in A2780 cells. **A,** Western blot analysis of H3K9me1 and H3K9me2 levels in response to increasing concentrations of UNC0638 in A2780 cells 48 hours after treatment. Total histone H3 was used as a loading control. Representative blots from three independent experiments are shown. **B,** Effects of dosing schedule by combination treatment with G9Ai and 5-aza-CdR on *HERV-Fc1* expression. A2780 cells (2.5×10^5) were seeded in 100-mm dishes at day −1, treated with 400 nmol/L G9Ai, 100 nmol/L 5-aza-CdR, or their combinations according to the schedule shown at the top. Cells were harvested at day 5 after 5-aza-CdR treatment. *HERV-Fc1* expression levels were then assayed by quantitative RT-PCR using the expression levels of *TBP* as a loading control and normalized to the level of *HERV-Fc1* expression after 5-aza-CdR treatment alone. Values are presented as mean ± SEM of three independent experiments. A one-way repeated-measures ANOVA was used for statistical analysis. *, $P < 0.05$.

contrast, the synergistic effects were limited in PEO14 and OAW42 cells (Fig. 2C; Supplementary Fig. S3).

As further characterization of the effects by combination treatments, we next monitored changes of total cell counts upon treatments using a Coulter counter. In this experiment, A2780, CAOV3, PEO14, and OAW42 cells were cultured in 100-mm dishes and treated with 400 nmol/L G9Ai alone or in combination with 100 nmol/L 5-aza-CdR following the dosing schedules outlined above. The results showed that A2780 and CAOV3 cells exhibited significantly reduced total cell counts by combination treatment compared with untreated or single-compound–treated cells (Supplementary Fig. S4). Because this effect could be due to reduced cell proliferation rate or increased cell death, we next examined the cellular phenotypes by flow cytometry using an amine reactive viability dye and performed cell-cycle analysis to test these possibilities. The reduced total cell counts in A2780 and CAOV3 cells were associated with increased cell death by combination treatment (Fig. 2D), while cell-cycle parameters remained unchanged compared with 5-aza-CdR treatment (Fig. 2E). These data indicated that low dose of G9Ai and 5-aza-CdR could act synergistically by promoting cell death in A2780 and CAOV3 cells. To the contrary, no significant differences in total cell counts (Supplementary Fig. S4), percentages of dead cells (Fig. 2D),and cell-cycle parameters (Fig. 2E) were found between combination treatment and 5-aza-CdR–treated PEO14 and OAW42 cells. These data further confirmed that the two compounds have no synergistic effects on cell growth or inducing cell death in PEO14 and OAW42 cells.

Combination treatment with G9Ai and 5-aza-CdR synergistically upregulates viral defense genes in A2780 and CAOV3, but not in PEO14 and OAW42, cells

To investigate gene-expression changes underlying the synergistic antitumor effects of combination treatments, we sequenced total RNA in A2780, CAOV3, PEO14, and OAW42 cells after treatments. A *P* value of 0.05 and 2-fold expression change were used as cutoffs to identify differential expressed genes between treated and untreated cells from two independent experiments. In general, G9Ai treatment alone did not induce global gene-expression changes in the four ovarian cancer cell lines (Supplementary Fig. S5), with only a few genes significantly upregulated in A2780 and CAOV3 cells (Fig. 3A). Although genes upregulated by 5-aza-CdR and combination treatment largely overlapped, more genes were upregulated by combination treatment compared with 5-aza-CdR treatment alone in all four cell lines (Fig. 3A; Supplementary Figs. S5 and S6A–S6C). We therefore performed gene ontology analysis to identify the functions of genes that were uniquely upregulated by combination treatment. Interestingly, the immune response pathways, especially the interferon pathways, as cellular defense response to virus were overrepresented in the set of genes uniquely upregulated by combination treatments in A2780 and CAOV3 cells (Fig. 3B). We and others have previously shown that these viral defense genes can be upregulated by 5-aza-CdR treatment and are responsible for inducing apoptosis or inhibiting proliferation of cancer cells (9–11). Therefore, the expression status of a panel of 24 viral defense genes was examined after G9Ai, 5-aza-CdR, and combination treatment compared with untreated cells (Fig. 3C). Consistent with previous reports (9–11), 5-aza-CdR treatment upregulated these viral defense genes in a cell type–specific pattern (Fig. 3C; Supplementary Fig. S7A and S7B), whereas G9Ai treatment alone slightly increased the expression of these genes less than 2-fold relative to untreated cells (Fig. 3C; Supplementary Fig. S7A and S7B). These viral defense genes were further upregulated by the combination treatment (Fig. 3C; Supplementary Fig. S7A and S7B), suggesting that the synergistic antitumor effects by the combination treatment were contributed in part by the increased viral defense response in A2780 and CAOV3 cells. In contrast, the same analysis showed that the viral defense pathway were not further upregulated by combination treatments in PEO14 and OAW42 cells

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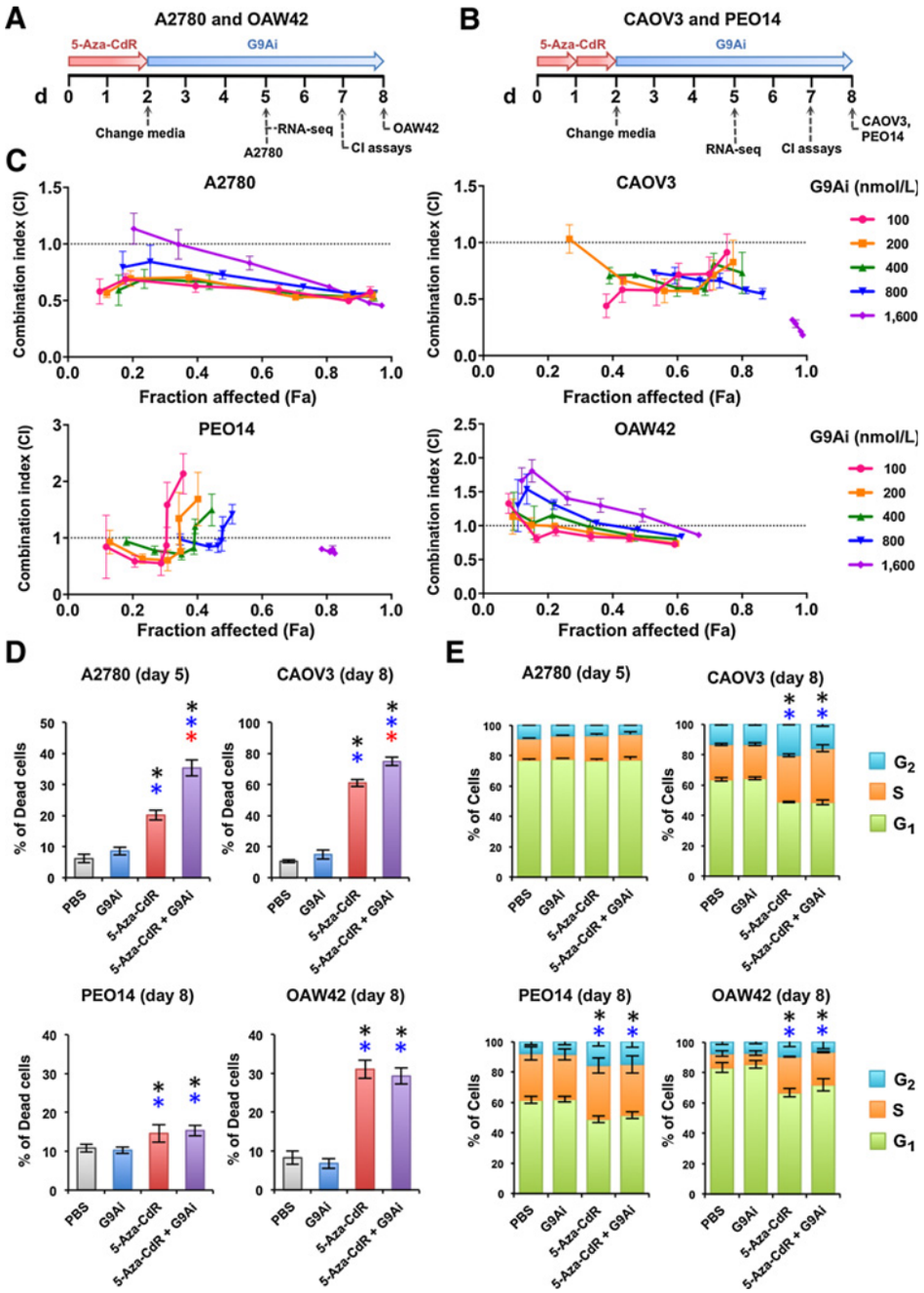

Figure 2.

Effects of combination treatment with 5-aza-CdR and G9Ai on proliferation, cell death, and cell cycle in A2780, CAOV3, PEO14, and OAW42 cells. **A,** Dosing schedule for the combination treatment in A2780 and OAW42 cells. **B,** Dosing schedule for the combination treatment in CAOV3 and PEO14 cells. **C,** Chou–Talalay model of the effects of combination treatment with varying concentrations of G9Ai and 5-aza-CdR. The fraction affected (Fa) values were determined using CellTiter-Glo luminescent cell viability assay as shown in Supplementary Fig. S3. CIs across the Fa values were calculated by CompuSyn software (ComboSyn, Inc.). CI < 1, CI = 1, and CI > 1 indicate synergism, additive effect, and antagonism, respectively. Values are presented as mean ± SEM of three independent experiments. **D,** Amine-based viability assay using Ghost Dye Violet 450 (Tonbo Biosciences) analyzed by flow cytometry. Values are mean ± SEM of three independent experiments. One-way repeated-measures ANOVA with Geisser–Greenhouse epsilon correction was used for statistical analysis. *, $P < 0.05$; asterisks in black, blue, and red indicate comparison with untreated, G9Ai-treated, and 5-aza-CdR–treated samples, respectively. **E,** Bar graphs show the percentages for G_1, S, and G_2–M cells by PI staining and analyzed by flow cytometry. Values are mean with 95% CI from three independent experiments. Beta mixed-effects regression with a random intercept to account for experimental (batch) differences were used to compare the differences between cell phases and treatments. FDR multiple testing adjustments were performed to account for multiple testing. R v3.4.1 was used to fit these regressions (https://cran.r-project.org/). *, $P < 0.05$ for all cell-cycle phases. Asterisks in black and blue indicate comparison with untreated and G9Ai-treated samples, respectively.

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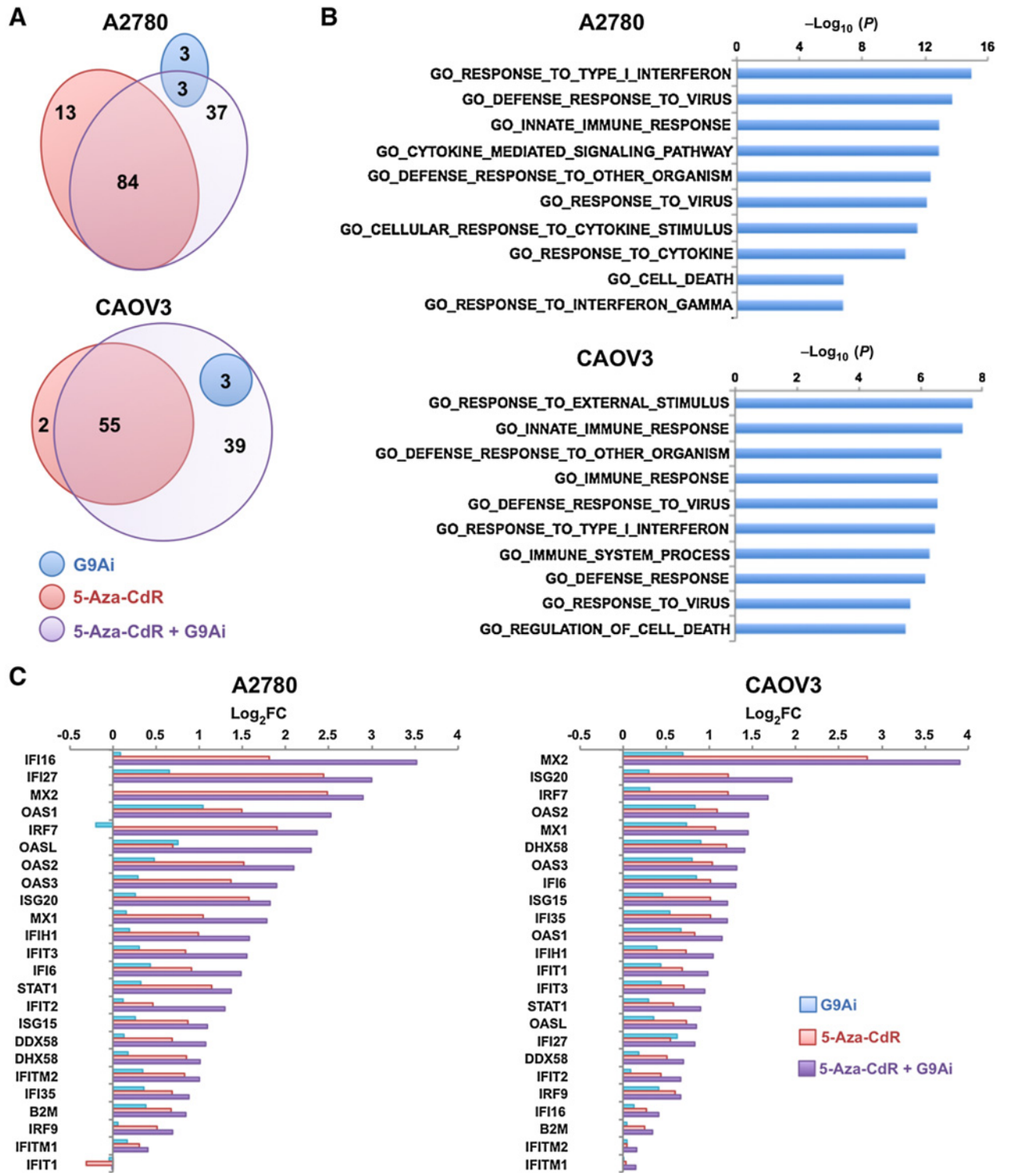

Figure 3.

Combination treatments further upregulated viral defense genes in A2780 and CAOV3 cells. **A,** Venn diagrams show the upregulated genes (greater than 2-fold change, $P < 0.05$) after G9Ai, 5-aza-CdR, and combination treatment compared with untreated cells. **B,** Bar graphs show the top 10 Gene Ontology Biological Process terms of genes uniquely upregulated by combination treatment (greater than 2-fold change, $P < 0.05$). **C,** Bar graphs show the expression fold change (in $\log_2$ values) of 24 viral defense genes as previously published (9, 10) in A2780 and CAOV3 cells after G9Ai, 5-aza-CdR, and combination treatment compared with untreated cells.

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(Supplementary Figs. S6B–S6C and S7C–S7D), in which no synergy was found (Fig. 2C–E). This observation strengthens our conclusion that the synergistic effects between G9Ai and 5-aza-CdR are associated with further induction of the viral defense pathway.

ERVs were upregulated by G9Ai, 5-aza-CdR, and combination treatment in a cell line–specific pattern

Because induction of ERV expression, especially as dsRNA, is key for triggering the viral defense response by 5-aza-CdR (9–11), we next tested whether ERVs were further upregulated by combination treatment. We mapped transcripts unique to ERV loci in two replicates of the RNA-seq data for all four cell types after the various treatment regimens. ERVs located within coding genes (including introns) were removed from analysis to focus on expression induced by their LTRs rather than as part of host genes. G9Ai treatment alone increased the total reads of intergenic ERVs in CAOV3 but not in A2780 cells (Fig. 4A). Although 5-aza-CdR increased the total intergenic ERV counts compared with untreated A2780 and CAOV3 cells, a further increase after combination treatment was found (Fig. 4A).

Our previous data also showed that some ERVs could be bidirectionally transcribed and that these overlapping transcripts can pair to form dsRNA (10). In addition, dsRNA are preferred substrates for the RIG-I–like receptors, such as RIG-I or MDA5, and are important inducers of antiviral immunity (9, 11, 47). We therefore analyzed the strand-specific RNA-seq data and detected increases in bidirectional transcribed ERVs mainly by 5-aza-CdR and combination treatment, similar to total intergenic ERVs (Fig. 4B). G9Ai treatment alone, however, significantly increased the total counts of the bidirectionally transcribed ERVs in both A2780 and CAOV3 cells (Fig. 4B). These data support a correlation between total bidirectionally transcribed ERVs and the upregulation of viral defense genes (Figs. 3C and 4B). Therefore, combination treatment of G9Ai and 5-aza-CdR enhanced the previously reported "viral mimicry" response by 5-aza-CdR alone, with increased total ERV counts, especially the dsERV counts, that further upregulated an antiviral immune response in these cells.

Interestingly, we observed that G9A and DNA methylation appears to repress a distinct set of ERVs, because the majority of ERVs upregulated by G9Ai treatment did not overlap with those upregulated by 5-aza-CdR (Fig. 4C). This is consistent with our recent finding that there is a switch in silencing mechanisms depending on the evolutionary age of ERVs (48). Evolutionary "young" ERVs tend to be CpG-rich and are repressed mainly by DNA methylation, while "intermediate aged" ERVs with lower CpG density are repressed predominantly by histone modifications, particularly H3K9 methylation (48). The combination treatment of G9Ai with 5-aza-CdR resulted in the activation of more ERVs than single-compound–treated A2780 and CAOV3 cells (Fig. 4C; Supplementary Fig. S8), the majority of which were uniquely upregulated by the combination treatment (Fig. 4C). These data suggest that these ERVs might be silenced by both G9A and DNA methylation. Alternatively, an "epigenetic switch" might happen at these ERVs, which become silenced by histone methyltransferases such as G9A upon loss of DNA methylation. Similar mechanisms have been described previously (18, 48–50). We also observed that different ERVs were upregulated in A2780 and CAOV3 cells, showing a cell line–specific pattern of ERV upregulation (Fig. 4D). Using quantitative RT-PCR, we confirmed that *HERV-Fc1* and *MLT1N2* were robustly upregulated by 5-aza-CdR and further by combination treatments in both cells (Fig. 4D; Supplementary Fig. S9A and S9B). However, *LTR12C*, a family of ERVs previously found to be induced by 5-aza-CdR treatment (10, 51), was not further upregulated by combination treatment (Supplementary Fig. S9A and S9B).

In PEO14 and OAW42 cells, however, no further increases in intergenic ERVs or bidirectionally transcribed ERVs were found with combination treatments compared with 5-aza-CdR–treated cells (Supplementary Fig. S10A and S10B), which might explain why no further upregulation of the viral defense genes is elicited by combination treatments. In summary, the cell lines exhibited variable responses to the combination treatments with respect to ERV upregulation.

G9Ai treatment does not alter DNA methylation levels at LINE-1 elements and 5′LTR of *HERV-Fc1*

To address the cell line–dependent effects on ERV upregulation by the combination treatments, we next examined the effectiveness of 5-aza-CdR in inhibiting DNA methylation. We previously observed that 5-aza-CdR treatment in HCT116 cells with stable knockdown of G9A causes a decrease in DNA methylation levels at the promoters of the G9A-targeted genes (37). We therefore used a MethyLight assay (43) to determine DNA methylation level at LINE-1 elements, an approximate measure of global DNA methylation levels. DNA methylation was not inhibited by G9Ai alone, but was more strongly inhibited by 5-aza-CdR in A2780 and CAOV3 than in PEO14 and OAW42 cells (Fig. 5A; Supplementary Fig. S11). No synergy in inhibition of DNA methylation was seen in any of the combination treatments (Fig. 5A). We then performed bisulfite sequencing to examine the DNA methylation levels at the 5′ LTR of *HERV-Fc1*, which serves as its promoter (illustrated in Fig. 5B), because the expression levels of *HERV-Fc1* were further upregulated by combination treatment compared with 5-aza-CdR treatment alone in A2780 cells (Fig. 1B). Consistent with the MethyLight assay results, bisulfite sequencing data showed no robust changes in DNA methylation induced by G9Ai treatment alone or in combination with 5-aza-CdR in A2780 cells (Fig. 5C). Thus, the enzymatic activity of G9A was dispensable for the maintenance or restoration of DNA methylation status at LINE-1 elements or the LTR of *HERV-Fc1* after 5-aza-CdR treatment. To summarize, although DNA demethylation was required for upregulation of a group of ERVs such as *HERV-Fc1*, the synergistic effects of the combination treatment to further upregulate these ERVs did not involve further DNA demethylation by G9Ai.

Combination treatment further reduces the level of chromatin-bound G9A protein and global levels of H3K9me1/2/3 in A2780 cells

Reduction of global and gene locus–specific G9A protein and H3K9 dimethylation levels by 5-aza-CdR treatment alone has been reported previously (30–32). Because the G9Ai (UNC0638) acts as a competitive inhibitor, the combination treatment with G9Ai and 5-aza-CdR could further reduce the chromatin-bound G9A protein and H3K9 methylation levels to increase promoter activity synergistically. To test this possibility, we performed chromatin association assays to quantify chromatin-bound G9A protein levels at day 5 after the treatments in A2780 cells. G9Ai treatment alone did not significantly change the chromatin-bound G9A protein levels (Fig. 6A and B), but increases free G9A proteins in both whole-cell and soluble fractions (Supplementary Fig. S12A–S12C). 5-Aza-CdR treatment resulted in a significant

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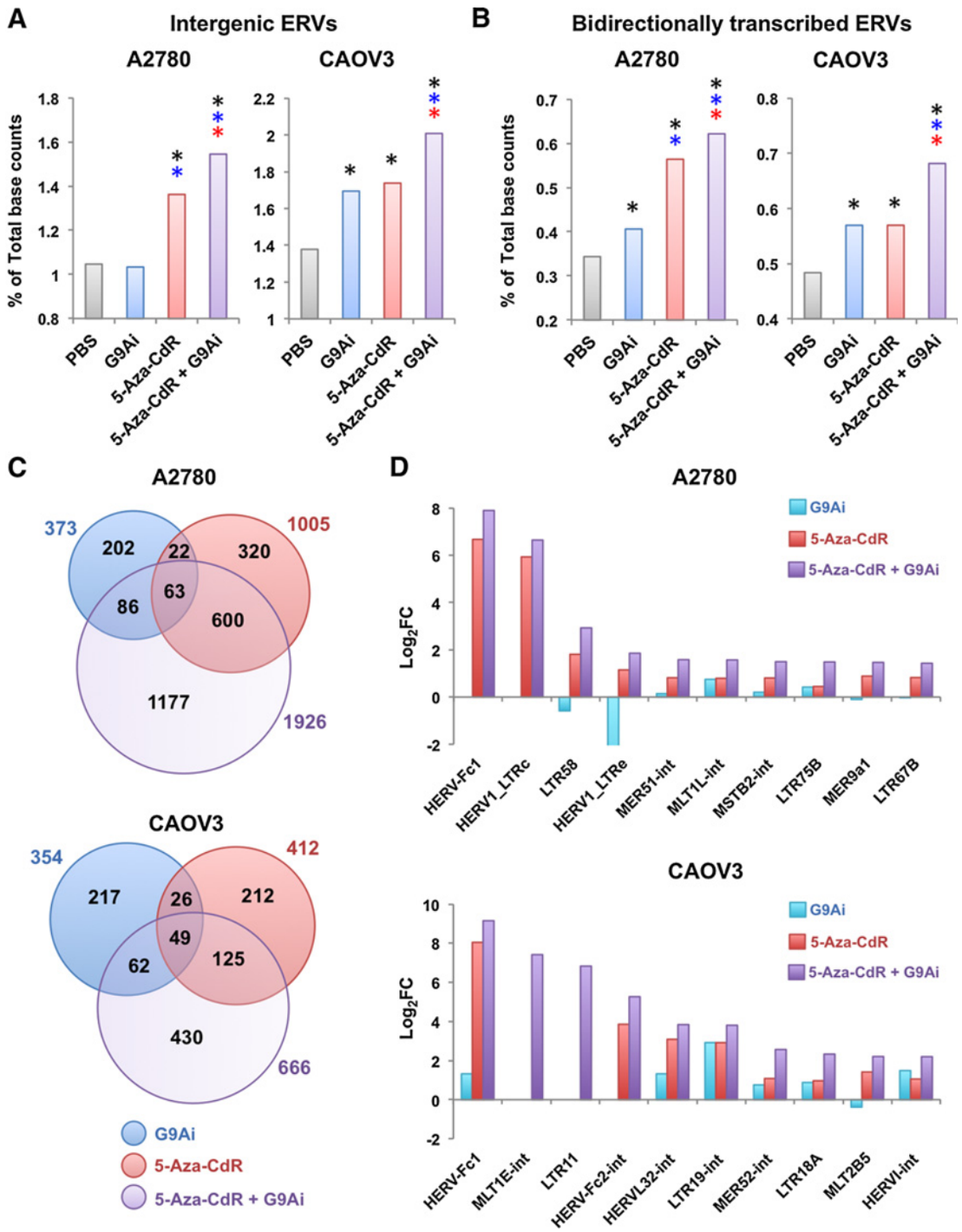

Figure 4.

Combination treatments further upregulated ERVs in A2780 and CAOV3 cells. **A,** Bar graphs show percentage of ERV base counts mapped uniquely to the intergenic region relative to total base counts in two independent RNA-seq experiments. *, $P < 0.05$ by χ^2 test for equality of proportions without continuity correction. **B,** Bar graphs show percentage of bidirectionally transcribed ERV base counts mapped uniquely to the intergenic region relative to total base counts in two independent RNA-seq experiments. *, $P < 0.05$ by χ^2 test for equality of proportions without continuity correction. Asterisks in black, blue, and red indicate comparison with untreated, G9Ai-treated, and 5-aza-CdR–treated samples, respectively. **C,** Venn diagrams show the upregulated ERVs (greater than 2-fold change, $P < 0.05$) after G9Ai, 5-aza-CdR, and combination treatment compared with untreated cells. **D,** Top 10 most upregulated ERV families in A2780 and CAOV3 cells. Values are $\log_2$-fold change of transcripts uniquely mapped to individual ERV family after G9Ai, 5-aza-CdR, and combination treatment compared with untreated cells.

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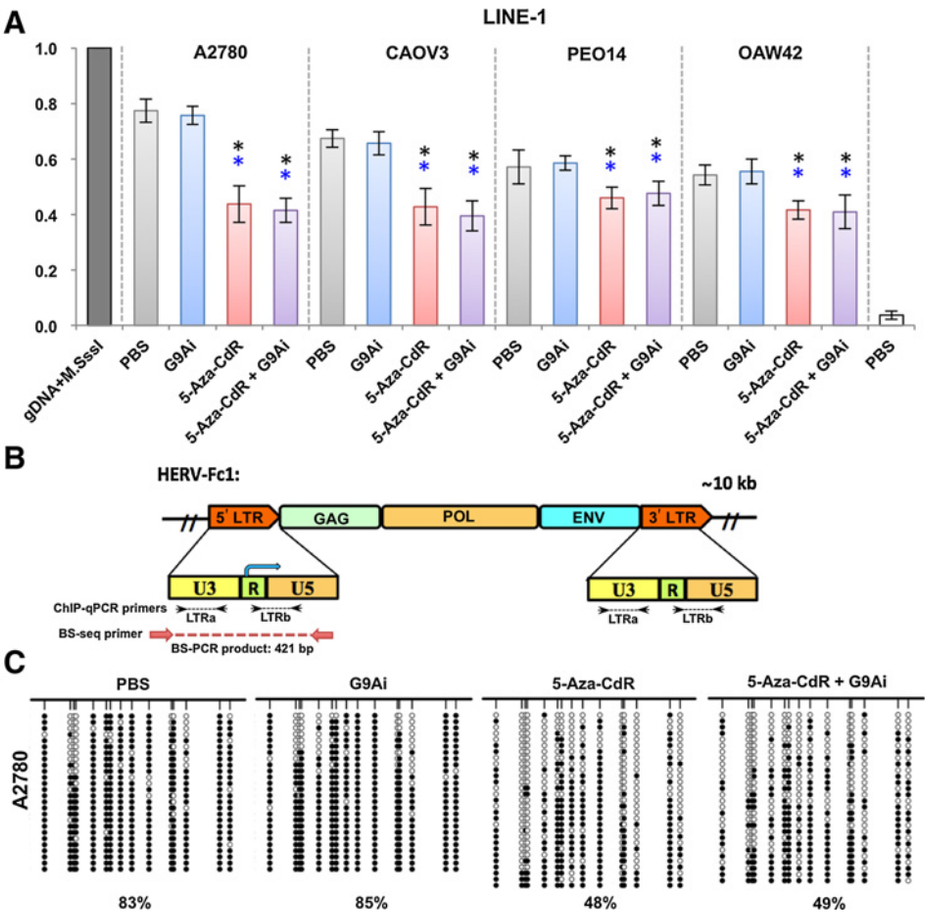

Figure 5.

DNA methylation levels at LINE-1 elements and the 5′LTR of *HERV-Fc1* before and after treatments. **A,** MethyLight analysis of DNA methylation at LINE-1 elements in A2780, CAOV3, PEO14, and OAW42 cells before and after treatment. MethyLight assay was performed using primers against methylated LINE-1 elements in bisulfite-converted DNA from these cells, alongside genomic DNA from DKO1 cells as a negative control. Methylation levels were calculated in triplicate by the $\Delta\Delta C_t$ method using unbiased *Alu* reactions as copy-number controls for each sample and normalized to fully methylated reference DNA (M.SssI-treated genomic DNA). Values are mean ± SEM, and one-way repeated-measures ANOVA was used for statistical analysis. *, $P < 0.05$; asterisks in black, blue, and red indicate comparison with untreated, G9Ai-treated, and 5-aza-CdR–treated samples, respectively. **B,** A schematic diagram of *HERV-Fc1* showing the structure of LTRs and the locations of primers for bisulfite sequencing and ChIP-qPCR. **C,** Bisulfite sequencing of the 5′LTR of *HERV-Fc1* at day 5 after treatments in A2780 cells. Bubble maps show the methylation status of cells before and after treatment, with each row representing a different read and each column representing a different CpG position. Filled and open circles indicate methylated and unmethylated CpG sites, respectively. The numbers below each bubble map show the respective CpG methylation percentages.

reduction of chromatin-bound G9A protein levels, which were further reduced by combination treatment in A2780 cells (Fig. 6A and B), despite the fact that more soluble G9A proteins were found after combination treatment than 5-aza-CdR treatment alone (Supplementary Fig. S12A and S12C).

We next quantified the levels of H3K9 methylation by Western blot in A2780 cells, and we found that both G9Ai and 5-aza-CdR significantly reduced global H3K9me1/2/3 levels, especially for H3K9me2, and the combination treatment further reduced the global levels of these repressive marks (Fig. 6C and D). Interestingly, global H3K9me3 levels were also reduced by the treatments in A2780 cells (Fig. 6C and D). Although the G9A/GLP complex does not catalyze trimethylation of H3K9, as the levels of H3K9me1/2 decreased, other lysine methyltransferase activities might have been reduced by depleting their substrates. Taken together, we observed a global reduction of chromatin-bound G9A protein and H3K9me1/2/3 levels by combination treatment compared with treatment with either compound alone, which might serve as the underlying mechanism for the synergistic effects of combination treatments in A2780 cells.

We also examined the H3K9me1/2 levels in OAW42 cells, in which no synergy was found between G9Ai and 5-aza-CdR. Interestingly, the results showed that 5-aza-CdR treatment alone efficiently reduced H3K9me1/2 levels to less than 20% of those in untreated cells (Supplementary Fig. S13A and S13B), compared with about 75% in A2780 cells (Fig. 6C and D). The combination treatment only slightly reduced the H3K9me1/2 levels compared with those after 5-aza-CdR treatment alone (Supplementary Fig. S13A and S13B). These data suggested that in OAW42 cells, 5-aza-CdR treatment is sufficient to remove most of the repressive H3K9me1/2 marks. The addition of G9Ai therefore might not be effective in further removing these marks globally. A search in our

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RNA-seq data revealed that the A2780 and CAOV3 cells showed higher levels of *G9A/GLP* expression, while in the cells that did not respond synergistically, lower levels of *G9A/GLP* transcripts were found (Supplementary Fig. S13C). These data suggested that the synergy between G9Ai and 5-aza-CdR might be, in part, associated with *G9A/GLP* expression levels.

Combination treatment further decreases H3K9me2 levels at LTRs of ERVs in A2780 cells

The RNA-seq data revealed that a cluster of ERVs could be further upregulated by combination treatments, making it likely that the LTRs of these ERVs were suppressed by H3K9me1/2 in addition to DNA methylation. Although our results demonstrated strong decreases in global H3K9me2 by G9Ai or combination treatment (Fig. 6C and D), it was still not clear whether these changes also occur at ERV LTRs. We therefore performed ChIP assays, followed by quantitative PCR (ChIP-qPCR) to detect the chromatin modification status at the LTRs of *HERV-Fc1* and *MLT1N2*, as two examples in A2780 cells. There was enrichment of the repressive mark H3K9me2 at LTRs of these ERVs compared with the promoters of β-actin (*ACTB*) (Fig. 6E). Indeed, the repressive H3K9me2 mark was dramatically reduced by G9Ai treatment alone. 5-Aza-CdR treatment resulted in increases, rather than decreases, of H3K9me2 levels at LTRs of these ERVs (Fig. 6E), suggesting an "epigenetic switch" to silencing the ERVs by histone modifications upon loss of DNA methylation (48, 50). H3K9me2 levels were further reduced by the combination treatment at LTRs of these ERVs, associated with an increase in active marks H3K4me3 and H3K27ac (Fig. 6E). Taken together, DNA demethylation by 5-aza-CdR and the additional chromatin alterations at ERV LTRs (decreasing H3K9me2 and increasing H3K4me3 and H3K27ac) induced by the combination treatment likely contributes to the synergistic upregulation of ERVs.

Discussion

Our study shows that inhibiting both DNA methylation and the histone methyltransferase G9A synergistically induces antitumor effects and enhances the "viral mimicry" response in ovarian cancer cells with high levels of G9A/GLP expression. Importantly, we have shown that *G9A* was overexpressed in primary ovarian tumors, and was the highest among 33 TCGA cancer types. In addition, overexpression of *G9A* has been associated with poor prognosis of EOC (29). Therefore, a treatment strategy targeting both G9A and DNA methylation provides a promising treatment option because DNA methylation inhibitors have already been tested in clinical trials with promising outcomes (52, 53). Our dual inhibition approach suggests a potentially new epigenetic therapy combination that may serve as a novel therapeutic strategy for patients with ovarian cancer.

A key mechanism underlying the synergistic effects of G9Ai and 5-aza-CdR on ERV upregulation may involve the "epigenetic switch," in which a group of ERVs were repressed by G9A upon loss of DNA methylation. This is in line with recent work from our laboratories that there is a gain in repressive marks such as H3K9me2/3 or H3K27me3 after DNA demethylation to maintain the silencing of some ERV LTRs (48–50). Because H3K9me2 levels at LTRs of *HERV-Fc1* and *MLT1N2* increased after 5-aza-CdR treatment, this histone mark may well be responsible for repressing these ERVs. This is different from the situation at promoters of protein coding genes where 5-aza-CdR effectively reduced H3K9me2 levels and depleted G9A (31, 32). Therefore, the addition of G9Ai after 5-aza-CdR treatment could enhance the expression of these ERVs by further reducing the repressive H3K9me2 marks. It is worth noting that the "epigenetic switch" might be dependent on the expression levels of G9A/GLP or other silencing complexes such as the polycomb repressive complex 2 (PRC2; ref. 50). In addition, different responses to DNA demethylation by 5-aza-CdR might also contribute to the variable synergistic effects observed in these cell lines after the combination treatment.

An interesting finding of this study is that G9A and DNA methylation appears to repress distinct sets of ERVs. This is consistent with our recent finding that DNA methylation tends to predominate in silencing the evolutionarily younger ERVs, which have higher CpG densities, while histone methyltransferases target mainly the middle-aged ERVs with less CpG density (48). Therefore, the combination treatment results in upregulation of more ERVs that include those repressed by G9A or DNA methylation alone. For evolutionary "young" ERVs such as *HERV-Fc1* and *MLTN1*, DNA methylation plays a major silencing role, and demethylation by 5-aza-CdR can induce their expression while G9Ai treatment alone is not sufficient to upregulate these ERVs. However, they were still repressed by G9A after 5-aza-CdR treatment (termed as the "epigenetic switch" mentioned above). Combination treatment simultaneously removed both repressive mechanisms, resulting in further upregulation of these ERVs. In this way, combination treatment with the two inhibitors synergistically strengthens the "viral mimicry" response and leads to increased antitumor effects.

Our findings also show that not all ERVs were equal with respect to their abilities to activate the viral defense pathway. In CAOV3 cells, where G9Ai treatment alone upregulated equivalent numbers of ERVs as 5-aza-CdR (Fig. 4A–C), the levels of induced viral defense genes were lower than those upregulated by 5-aza-CdR treatment (Fig. 3C). This observation reinforces our previous findings that the intermediate-age ERVs silenced mainly by histone methyltransferases did not elicit a strong antiviral response (48). The strength of the viral mimicry response may be determined by the expression of evolutionary "younger" ERVs silenced by DNA methylation. Further studies are needed to characterize the effects of specific ERVs in inducing the antiviral response, which may be crucial for patient responses to the combination epigenetic therapy.

Our results also suggest that G9A plays different roles in various tissues or cell types. For example, it has been shown that G9A is essential in repressing developmental genes at euchromatic regions during early embryogenesis (54). In breast cancer cells, G9A has been shown to be responsible for aberrant silencing of tumor suppressor genes (30). In our study, G9Ai treatment alone did not induce upregulation of tumor suppressor genes. In fact, very few genes were upregulated; instead, a subset of ERVs were upregulated by G9Ai treatment alone in some ovarian cancer cells, highlighting the important role of G9A in suppressing ERVs in ovarian cancer cells.

UNC0638 was designed based on the structure of G9Ai BIX01294 with improved *in vitro* potency and increased cell membrane permeability (46). UNC0638 exhibited high potency and specificity for inhibiting G9A and GLP with very low off-target toxicities (46). However, its potential for clinical application is limited by poor pharmacokinetics *in vivo*. The development of next generation G9Ai with comparable potency is needed to improve *in vivo* pharmacokinetics. Our current preclinical study

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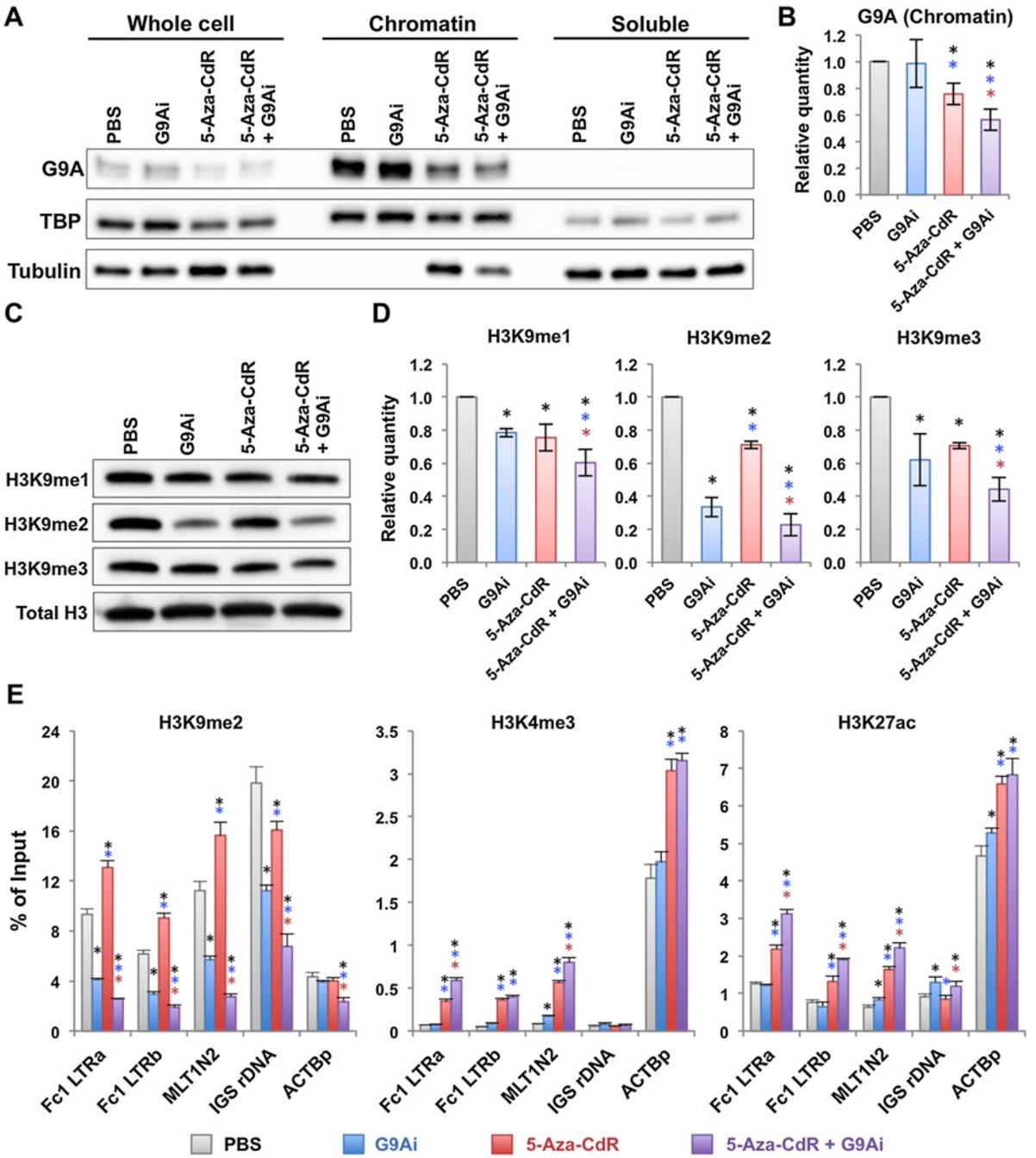

Figure 6.

Changes in G9A protein and histone modification levels in A2780 cells at day 5 of the treatment schedule. **A,** Western blot analysis of G9A protein levels in whole-cell, chromatin, and soluble fractions. TBP and β-tubulin proteins were used as controls. Representative blots are shown from three independent experiments. **B,** Bar graph shows the quantification of the chromatin-associated G9A protein levels relative to TBP protein levels. The values were normalized to the levels of G9A protein before treatment and are represented as mean ± SEM in three independent experiments. One-way repeated-measures ANOVA with Geisser–Greenhouse epsilon correction was used for statistical analysis. *, $P < 0.05$; asterisks in black, blue and red indicate comparison with untreated, G9Ai-treated, and 5-aza-CdR–treated samples, respectively. **C,** Western blot analysis of total H3K9me1/2/3 modification levels in A2780 cells after treatments. Representative blots are shown from three independent experiments. **D,** Bar graphs show the quantification of H3K9me1/2/3 modification levels relative to total H3 levels and normalized to the levels in untreated samples in three independent experiments. Statistical analysis was performed as in **B**. **E,** Quantification of H3K9me2, H3K4me3, and H3K27ac levels by ChIP-qPCR at LTR regions of *HERV-Fc1* (Fc1 LTRa and b) and *MLT1N2*. The intergenic spacer region of the 35S ribosomal DNA genes (IGS rDNA) and the promoter region of β-actin (*ACTBp*) were used as controls. Values are mean ± SEM ($n = 3$). One-way repeated-measures ANOVA was used for statistical analysis. *, $P < 0.05$; asterisks in black, blue, and red indicate comparison with untreated, G9Ai-treated, and 5-aza-CdR–treated samples, respectively.

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has provided a rationale for the future clinical application using the combination of inhibitors for both G9A and DNMTs. We predict that this combination would be very helpful for those cancer types that exhibit overexpression of G9A or GLP proteins, especially for those cancers with poor prognosis correlated with higher *G9A* expression levels, such as ovarian cancer. Our results are limited to the four cell lines tested so that the generality of the results need to be replicated in a larger set. However, they are consistent with recent data we have obtained with knockdowns of histone methyltransferases and point to the need for patient stratification based on the levels of *G9A/GLP* before combination treatment is initiated.

Conclusions

DNA methylation and the histone modifications H3K9me1/2 are both important for silencing ERVs. Dual inhibition of these processes results in synergistic upregulation of ERVs and induces an antiviral response in some but not all cell lines, serving as a basis for exploring this novel combination treatment in the clinic, especially for patients with ovarian cancer with high levels of H3K9me1/2 histone methyltransferases *G9A/GLP* expression.

Disclosure of Potential Conflicts of Interest

P.A. Jones is a consultant/advisory board member for Zymo Corporation. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M. Liu, S.L. Thomas, A.K. DeWitt, G. Liang, P. A. Jones
Development of methodology: M. Liu, S.L. Thomas, H. Ohtani
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Liu, S.L. Thomas, A.K. DeWitt
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Liu, S.L. Thomas, A.K. DeWitt, W. Zhou, Z.B. Madaj, G. Liang
Writing, review, and/or revision of the manuscript: M. Liu, S.L. Thomas, A.K. DeWitt, Z.B. Madaj, S.B. Baylin, G. Liang, P. A. Jones
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Liu, S.L. Thomas, A.K. DeWitt, W. Zhou
Study supervision: G. Liang, P. A. Jones
Other (planning of experiments): S.B. Baylin

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Dual Inhibition of DNA and Histone Methyltransferases Increases Viral Mimicry in Ovarian Cancer Cells

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