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PRINCIPAL INVESTIGATOR: Dipanjan Chowdhury, PhD

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute
                                                    Boston, MA 02215

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E-Mail: Dipanjan_Chowdhury@dfci.harvard.edu	

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14. ABSTRACT

Approximately 20% of high grade serous ovarian cancers harbor Cyclin E1 (CCNE1) amplification and are associated with poor outcome and inferior responsiveness to standard platinum chemotherapy. Given their intrinsic resistance to platinum, management of CCNE1-amplified ovarian cancers is challenging. In this research, we evaluate three novel strategies against CCNE1-amplified ovarian cancers that address different aspects of CCNE1 biology. In the first aim, based on our preliminary data, we hypothesize that HSP90-inhibitors may be effective against CCNE1-amplified ovarian tumors because they suppress HR, downregulate BRCA1, and downregulate CCNE1. In the second aim, based on our preliminary data and the fact that RB functions downstream of cyclin E, we hypothesize that inhibition of FOXM1 and RB interaction is an effective approach for targeting CCNE1-amplified ovarian tumors. Specifically, suppression of FOXM1/RB interaction will lead to enhancement of RB/E2F interaction and suppression of E2F-dependent oncogenic activity resulting in activity against CCNE1-amplified cells. In the third aim, we hypothesize that miR-1255b, miR-148b*, and miR-193b* may be effective against CCNE1-amplified ovarian tumors in combination with platinum and PARPis. Potential mechanisms for this effect include suppression of HR and downregulation of BRCA1, RAD51 and BRCA2 that are relevant for CCNE1-amplified ovarian tumors which are dependent on hyperactive HR and are sensitive to suppression of BRCA1.

15. SUBJECT TERMS
Ovarian Cancer, CCNE1 amplification, Homologous Recombination, Platinum analogues, MicroRNAs, Heat shock protein 90 inhibitors, Forkhead box protein M1, Retinoblastoma

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INTRODUCTION:

Approximately 20% of high grade serous ovarian cancers harbor Cyclin E1 (CCNE1) amplification and are associated with poor outcome and inferior responsiveness to standard platinum chemotherapy. Given their intrinsic resistance to platinum, management of CCNE1-amplified ovarian cancers is challenging. In this research, we evaluate three novel strategies against CCNE1-amplified ovarian cancers that address different aspects of CCNE1 biology. In the first aim, based on our preliminary data, we hypothesize that HSP90-inhibitors may be effective against CCNE1-amplified ovarian tumors because they suppress HR, downregulate BRCA1, and downregulate CCNE1. In the second aim, based on our preliminary data and the fact that RB functions downstream of cyclin E, we hypothesize that inhibition of FOXM1 and RB interaction is an effective approach for targeting CCNE1-amplified ovarian tumors. Specifically, suppression of FOXM1/RB interaction will lead to enhancement of RB/E2F interaction and suppression of E2F-dependent oncogenic activity resulting in activity against CCNE1-amplified cells. In the third aim, we hypothesize that miR-1255b, miR-148b*, and miR-193b* may be effective against CCNE1-amplified ovarian tumors in combination with platinum and PARPis. Potential mechanisms for this effect include suppression of HR and downregulation of BRCA1, RAD51 and BRCA2 that are relevant for CCNE1-amplified ovarian tumors which are dependent on hyperactive HR and are sensitive to suppression of BRCA1.

1. KEYWORDS:

Epithelial Ovarian Cancer, CCNE1 amplification, Homologous Recombination DNA Repair, Platinum analogues, MicroRNAs, Heat shock protein 90 (HSP90) inhibitors, Forkhead box protein M1 (FOXM1), Retinoblastoma (RB), Poly-ADP Ribose Polymerase Inhibitors (PARP-inhibitors)

2. ACCOMPLISHMENTS:

What were the major goals and objectives of the project?

The major goal for Aim 1 is to determine the activity of HSP90 inhibitors in CCNE1-amplified ovarian tumors.
The major goal for Aim 2 is to inhibit FOXM1 and RB interaction to suppress CCNE1-amplified ovarian tumors.
The major goal for Aim 3 is to determine the activity of certain miRNA mimics in combination with PARP-inhibitors or platinum against CCNE1-amplified ovarian tumors.

What was accomplished under these goals?

Since the starting of the award, substantial progress has been made toward achieving the goals as outline in the application.

For AIM 1:

The major goal for Aim 1 was to determine the activity of HSP90 inhibitors in CCNE1-amplified ovarian tumors. During the duration of the award we were able to achieve the following goals:

1) *The HSP90-inhibitor 17-AAG has single agent activity against a number of CCNE1-amplified cell lines*

As proposed in Aim 1, we evaluated the activity of HSP90-inhibitor 17-AAG in various CCNE1-amplified ovarian cancer lines, including OVCAR3, COV318 and in the OVCAR4 cell line which harbors CCNE1 overexpression. To that end, cells were plated at 1000 cells per well on a 96- well plate in sextuplicate and treated with 17-AAG at indicated concentrations on the next day. After 5 days, cell viability was quantified by Celltiter Glo.

As shown in Figure 1A, we noted significant activity of 17-AAG in OVCAR3 and OVCAR4 cell lines with IC50 of 0.07 and 0.08uM respectively. 17-AAG was also active in COV381 with an IC50 of 0.14uM, albeit less than OVCAR3 and OVCAR4. This is consistent with our hypothesis that HSP90 inhibitors may have good single agent activity against CCNE1 amplified and overexpressing lines.
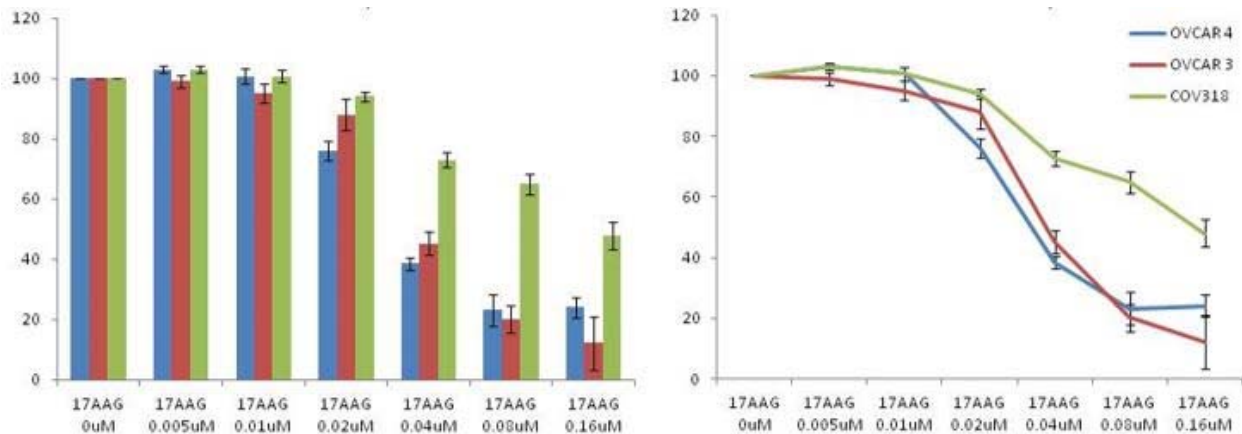

Figure 1. Activity of 17-AAG in OVCAR3, COV318 CCNE1 amplified and OVCAR4 CCNE1 overexpressing ovarian cancer lines.

2) *HSP90-inhibition downregulates homologous recombination (HR) DNA repair*

One of our hypothesis of how HSP90 may have activity against CCNE1 amplified cells is based on the fact that CCNE1 amplified cells are hyperdependent on an intact HR. Therefore, by downregulating HR, HSP90 inhibitors may induce lethality in CCNE1 amplified cells. In this regard, we assessed whether the HSP90 inhibitor AT13387 suppresses HR using the Direct Repeat-GFP (DR-GFP) reporter assay [1]. In this assay, there is measurement of HR-mediated repair of an I-SceI induced site specific DSB. Specifically, 0.1 X 106 U2OS cells carrying DR-GFP reporter were plated on a 12-well plate overnight, treated with 17 AAG at indicated concentrations for 24 hrs, and transfected with 500 ng of I-SceI expression plasmid or control vector using Lipofectamine 2000. After 48 h, GFP-positive cells were assayed by FACScan.

As shown in Figure 2, increasing concentrations of AT13387 reduced the efficacy of HR DNA repair by approximately 70%, down to plateau of 30%. This was also confirmed using the RAD51 foci formation after ionizing radiation (IR) assay.
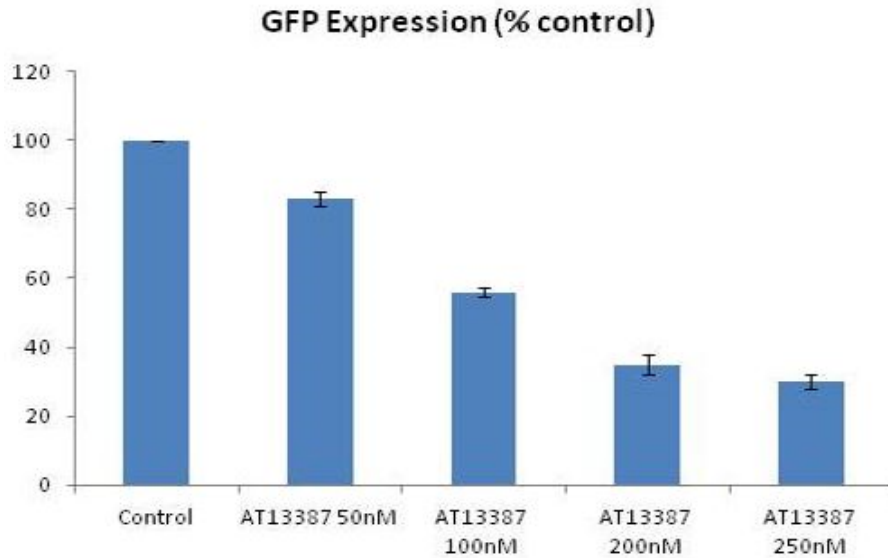
Figure 2. HSP90 inhibitor AT13387 suppresses HR as assessed by the Direct Repeat-GFP (DR-GFP) reporter assay.

3) _The HSP90-inhibitor AT13387 synergizes with DNA damage inducing agents such as PARP-inhibitors against CCNE1-amplified cell lines_

One of the key goals of Aim 1 was to also assess the activity of HSP90 inhibitors in combination with platinum and PARP inhibitors in CCNE1-amplified cell lines.

We assessed the combination of the novel PARP inhibitor BMN673 (talazoparib) in combination with the HSP90 inhibitor AT13387.

Figure 3. In vitro synergism between HSP90 inhibitor AT13387 and the PARP inhibitor BMN673 in OVCAR4 and OVCAR3.
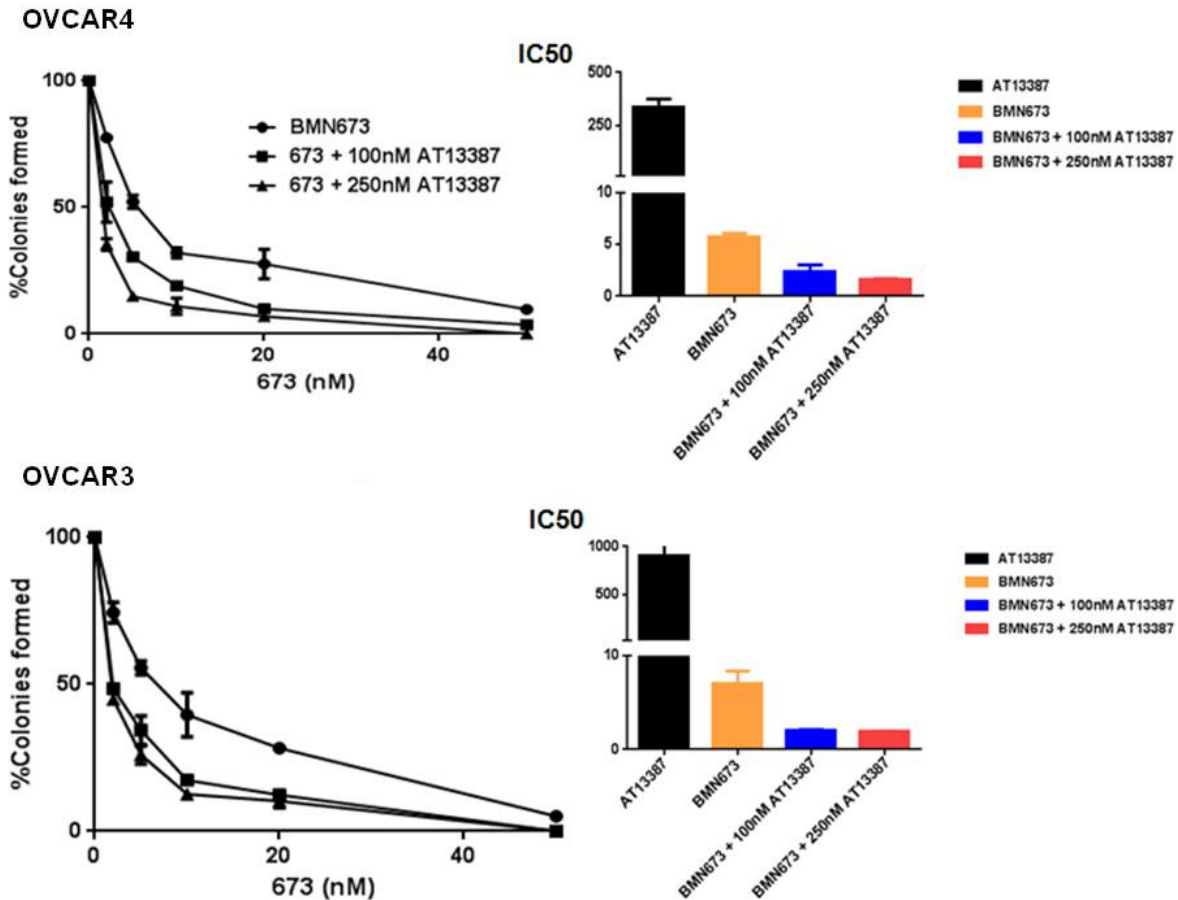
We have now assessed the combination talazoparib and AT13387 in OVCAR4 and OVCAR3 which exhibit CCNE1 overexpression and amplification respectively using colony formation assay. Cells were treated with the indicated (Figure 3) talazoparib and AT13387 concentrations. As shown in Figure 3, in both cell lines, addition of small concentrations of AT13387 enhanced the cytotoxicity of talazoparib, indicating synergism between the two drugs.

4. The HSP90-inhibitor AT13387 synergizes with PARP-inhibitors in CCNE1-amplified patient derived xenograft models

We assessed the effects of the combination of HSP90-inhibitors and PARP-inhibitors in patient derived CCNE1-amplified ovarian cancer cells that have been orthotopically propagated in immunocompromised mice.

Specifically, ovarian cancer cells have been taken from patients and implanted intraperitoneally into immunocompromised mice. These models have been luciferized thereby providing the ability to follow the growth of tumors and response to targeted therapies by non-invasive bioluminescent imaging (BLI) technology. Growth kinetics of

each model have been accurately determined using the BLI technology which allows reproducibility of model growth and accurate estimation of tumor burden which is important for randomization prior to drug testing. In addition, surrogate biomarkers such as CA125 have been evaluated as alternative methods to assess tumor burden and response to therapy.

We initially performed tolerability studies in three ovarian PDX models (DF101, DF106, DF09) using the PARP-inhibitor olaparib and the HSP90-inhibitor AT13387. As shown in Figure 4, doses of olaparib up to 100mg/kg po daily x 3 weeks and AT13387 up to 45mg/kg po for 2 days (D1,D2) on / 5 days off x 3 weeks (i.e. Days 1, 2, 8, 9, 15, 16) were well tolerated without weight loss in the mice.

Efficacy studies were subsequently performed; the study design is shown in Figure 5 below. Olaparib was dosed at 100mg/kg po daily x 4 weeks, AT13387 was administered at 45mg/kg po for 2 days (D1,D2) on / 5 days off x 4 weeks (i.e. Days 1, 2, 8, 9, 15, 16, 22, 23).
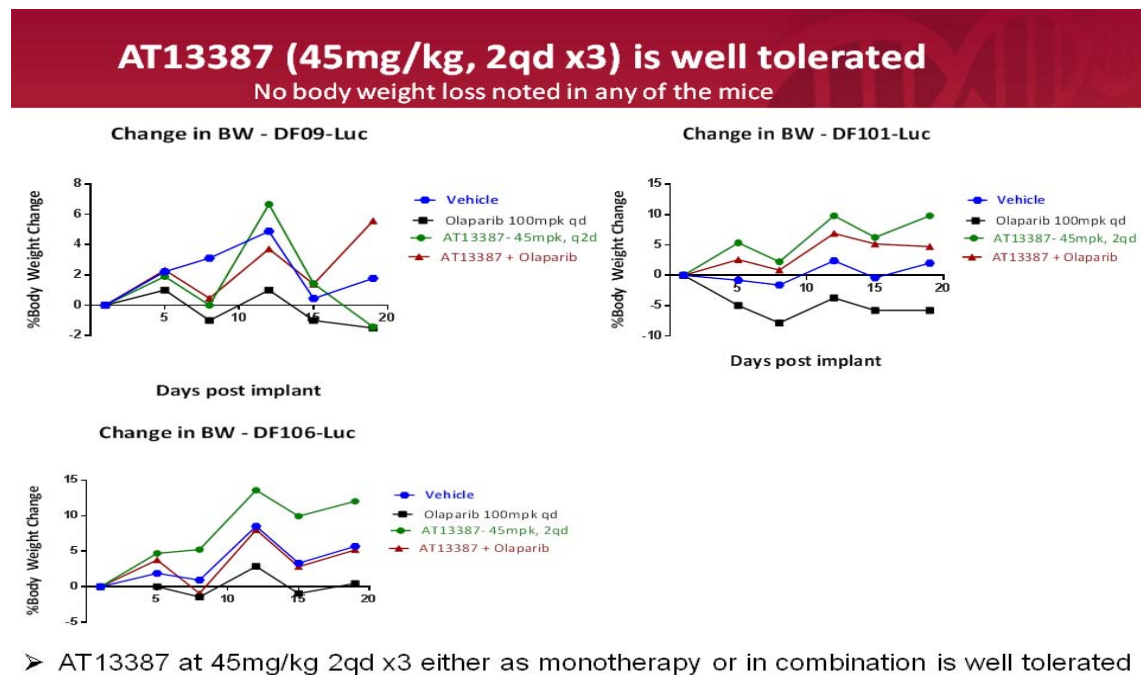
Figure 4. Tolerability of olaparib and AT13387 combination in ovarian PDX models.
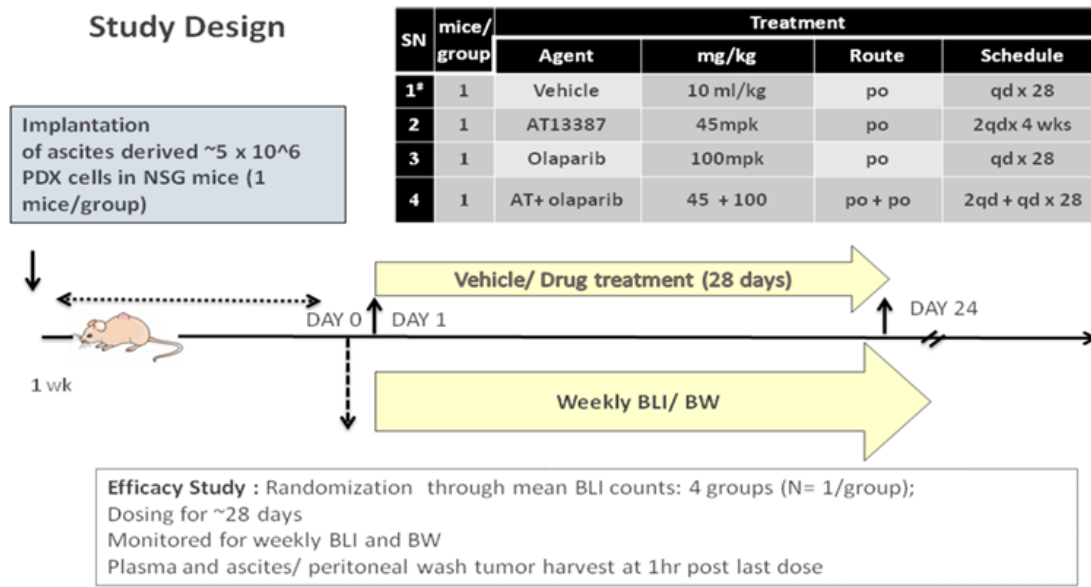
Figure 5. Design of efficacy studies of AT13387 and olaparib in ovarian PDX models.

We have now completed evaluation of all 4 CCNE1-amplified/overexpressed models and the results are presented in Figure 6 below. As shown in Figure 6, the combination of AT13387 and olaparib induced inhibition of tumor growth in the 4 CCNE1-amplified/overexpressed models. Of note, in these models, there was concomitant decrease in the CA125 biomarker using a mouse plasma CA125 assay determined serially (Figure 6).

Figure 6. AT13387 and olaparib induced inhibition of tumor growth in the 4 CCNE1-amplified/overexpressed ovarian cancer PDX models. Determination of the CA125 using a mouse plasma assay is also depicted for each model.

It is important to underscore that all these models were derived from patients with platinum and PARPi resistant tumors. These results support our hypothesis of Aim 1 that HSP90-inhibitors may sensitize tumors to PARP-inhibitors.
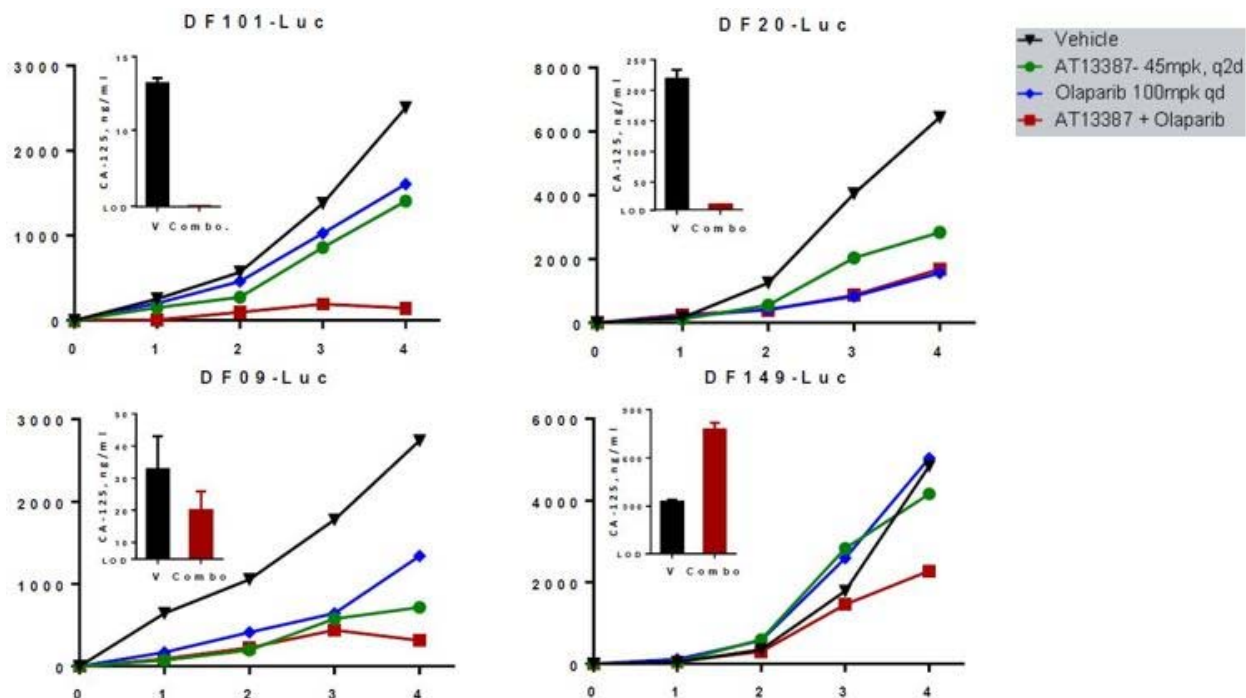
5. Initiation of a Phase I clinical trial of olaparib and AT13387 with dose expansion in ovarian cancer

We have initiated a Phase 1 Study of PARP inhibitor olaparib and HSP90 inhibitor AT13387 for treatment of advanced solid tumors with expansion in patients with recurrent epithelial ovarian, fallopian tube, peritoneal cancer or recurrent triple-negative breast cancer (CTEP/ETCTN protocol 10031 and NCI clinicaltrials.gov #NCT02898207). This is a multi-center, Phase 1/1b clinical trial with the objectives to define the MTD, RP2D and safety and tolerability of the combination of AT13887 and olaparib in patients with advanced solid tumor malignancies. The study will initially explore dose and regimen of the combination of these 2 novel agents. As viable regimens are identified, an expansion cohort of patients with epithelial ovarian, fallopian or primary peritoneal or triple negative breast cancer (TNBC) patients will be enrolled with one of the schedules, for confirmation of safety, PK and PD. This cohort will include patients with BRCA-wildtype TNBC or patients with platinum resistant epithelial ovarian or fallopian or primary peritoneal cancers.

As of today, the study is enrolling in dose escalation. The current dose level, DL3 (300mg BID olaparib + 80mg/m2 AT13387), has completed enrollment with 1 DLT of grade 3 anemia to date. Provided there are no further DLTs, we plan to accrue 3 additional patients on DL3 in the new year.

For AIM 2:

This aim focuses on targeting FOXM1 dependence in *CCNE1*-amplfied EOCs. The retinoblastoma protein (RB) is a tumor suppressor that functions downstream of cyclin E1 (encoded by the *CCNE1* gene) to regulate the cell cycle, apoptosis, and differentiation through its direct binding to and inhibition of the E2F transcription factor [2, 3]. Disruption of RB and E2F interaction by viral oncogenic proteins such as HPV-E7 leads to neoplastic transformation [4]. HPV-E7 inhibits RB function through a conserved LxCxE motif for high affinity RB bind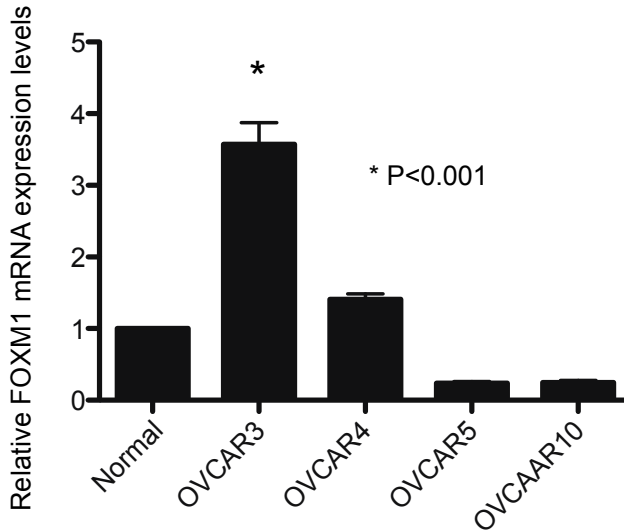ing [4, 5]. Although the RB pathway including its upstream regulator cyclin E is often deregulated in EOC [6], genetic alterations of the RB gene itself are relative rare in EOC [7-9]. Notably, RB physically interacts with FOXM1 [10, 11], a transcription factor with oncogenic activity in EOC [6]. Interestingly, the FOXM1 transcriptional network is significantly upregulated in EOC as well [6]. Therefore, we hypothesized that the interaction between RB and FOXM1 can be targeted in EOC. Since RB functions downstream of cyclin E, we expect that this approach will be especially effective in *CCNE1* amplified EOCs.

Figure 7. Expression of FOXM1 mRNA in a panel of EOC cell lines and normal fallopian tube epithelial cells determined by qRT-PCR.

We first examined the expression of *FOXM1* mRNA in a panel of EOC cell lines by qRT-PCR. We used normal fallopian tube epithelial cells as a control. Notably, *CCNE1*-amplified OVCAR3 showed the highest levels of FOXM1 expression (**Figure 7**). Thus, we used OVCAR3 cells to perform the subsequent functional studies. To determine the role of FOXM1 in OVCAR3 cells, we developed a shRNA targeting the human FOXM1 gene. We validated the knockdown efficiency for shFOXM1 by both qRT-PCR and immunoblotting (**Figure 8**).
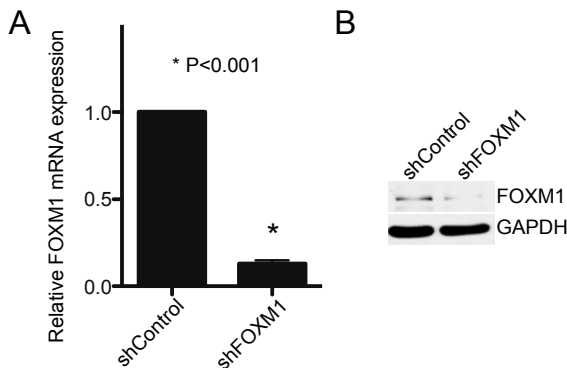

Figure 8. shRNA mediated knockdown of FOXM1 in OVCAR3 cells. Expression of FOXM1 in OVCAR3 expressing shFOXM1 or controls was determined by qRT-PCR (A) or immunoblotting (B).

Supporting the notion that FOXM1 is required for the proliferation of CCNE1 amplified EOC cells, FOXM1 knockdown significantly suppressed the growth of OVCAR3 cells as determined by both cell growth curve and colony formation assays (**Figure 9**).
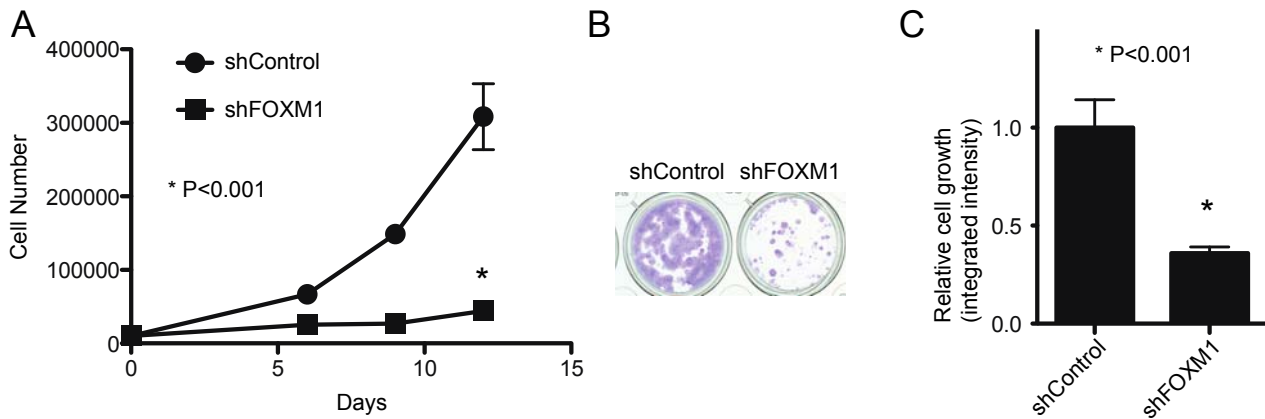
Figure 9. FOXM1 knockdown suppresses the growth of OVCAR3 cells. (A) Growth curves of OVCAR3 cells expressing shFOXM1 or controls. Mean of three independent experiments with SD. (B) Same as (A) but for colony formation assay. (C) Quantification of (B). Mean of three independent experiments with SD.
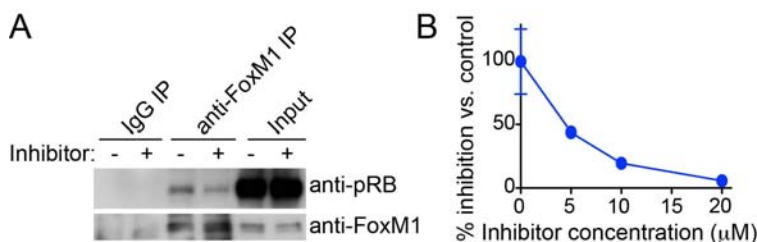

Figure 10. An inhibitor of FOXM1 and RB interaction that inhibits the growth of CCNE1 amplified NIH-OVCAR3 EOC cells. A) CCNE1 amplified NIH –OVCAR3 cells were treated with 5 mM inhibitor 478726 or control inactive compound 77333 for 48 h. The indicated cells were used for co-immunoprecipitation assay using an anti-FOXM1 antibody or a control IgG. The IPed product were examined by immunoblotting using the indicated antibodies. B) Same as A) but examined cell growth using the indicated concentration of inhibitor or control compound for 72 hours. Curves represent cell numbers normalized to control treatment.

The binding between FOXM1 and RB depends upon a LxCxE motif on FOXM1 [10, 11]. Notably, a class of thiazolidinedione compounds have previously been identified that disrupt the LxCxE motif mediated interaction between HPV-E7 and RB [12]. These compounds are selectively cytotoxic in HPV-positive cells *in vitro* and *in vivo* in mouse models [12]. The observed effects correlate with its ability to suppress the disruption of RB/E2F complex by HPV-E7. This leads to a restoration of RB/E2F interaction and suppression of E2F-dependent oncogenic activity. Since FOXM1's interaction with RB is also dependent upon the LxCxE motif [4, 5], we examined the effects of the RB/HPV-E7 disruption compound 478166 (or inhibitor 478726) on the interaction between FOXM1 and RB in EOC cells. In *CCNE1* amplified NIH-OVCAR3 cells [13], the interaction between FOXM1 and RB is readily detectable (**Figure 10A**). Co-immunoprecipitation analysis revealed that the interaction between FOXM1 and RB is substantially suppressed by inhibitor 478726 (**Figure 10A**). Since the disruption of interaction between HPV-E7 and RB by the inhibitor leads to cell growth arrest in HPV positive human cancer cells [12], we examined whether disruption of FOXM1 and RB also inhibits the growth of *CCNE1* amplified NIH-OVCAR3 cells. Indeed, we observed a dose-dependent suppression of cell growth by the inhibitor 478726 in these cells (**Figure 10B**).
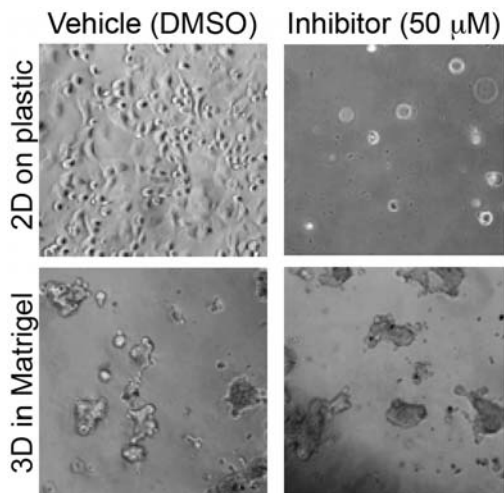
Figure 11. FOXM1 and Rb interaction inhibitor failed to suppress the growth of OVCAR3 cells in 3D culture in Matrigel. OVCAR3 cells were treated with the indicated concentration of the FOXM1 and Rb interaction inhibitor or vehicle DMSO control for 5 days cultured in 2D in plastic dishes or 3D in Matrigel.

We next thought to mimic the tumor microenvironment more closely by performing the analysis in three-dimensional culture given its role in determining the response to cancer therapeutics. Notably, the dose of 478726 that is effective in disrupting the RB and FOXM1 interaction was not effective in OVCAR3 cells. In summary, our data establish that FOXM1 is a valid therapeutic target in CCNE1 amplified EOCs. However, disrupting the interaction between FOXM1 and RB using small molecule inhibitors may not be sufficient in suppressing *CCNE1* amplified EOCs.

In summary, we have demonstrated in Aim 2:
1) FOXM1 is necessary for the proliferation of CCNE1 amplified epithelial ovarian cancer cells.
2) FOXM1 interacts with Rb in CCNE1 amplified epithelial ovarian cancer cells.
3) Characterized small molecule inhibitor that disrupts the interaction between FOXM1 and Rb in CCNE1 amplified epithelial ovarian cancer cells.
4) FOXM1 is a validate therapeutic target in CCNE1-amplified epithelial ovarian cancer cells.
5) Disruption of the interaction between FOXM1 and Rb by small molecule inhibitor may not be sufficient to suppress CCNE1 amplified EOCs.

For AIM 3:

The major goal for Aim 3 is to determine the activity of certain miRNA mimics in combination with PARP-inhibitors or platinum against CCNE1-amplified ovarian tumors. We were able to show the following:

1) Certain miRNAs including miR-1255b, miR-148b*, and miR-193b* inhibit HR DNA repair

As proposed in Aim 3, we evaluated whether certain miRNAs may inhibit HR repair in CCNE1 amplified cell lines. To achieve this we assessed the effects of miRNA mimics for miR-1255b, miR-148b*, and miR-193b* and miR-182 in CCNE1 amplified OVCAR3 cells using the RAD51 foci formation after ionizing radiation (IR) assay.
Specifically, OVCAR3 cells were transfected with control miRNA mimic and the indicated (Figure 12) miRNA mimics stained for RAD51 (green) and 4′,6-diamidino-2-phenylindole (DAPI) (blue) 6 h after exposure to IR. The images were captured by fluorescence microscopy and RAD51 focus-positive cells (with > 5 foci) were quantified by comparing 100 cells.

As shown in Figure 12, treatment with miRNA mimics for miR-1255b, miR-148b*, and miR-193b* and miR-182, significantly reduced RAD51 foci formation after IR compared to control miRNA mimic, suggesting that these miRNA mimics can indeed inhibit HR repair in these CCNE1 amplified cells.
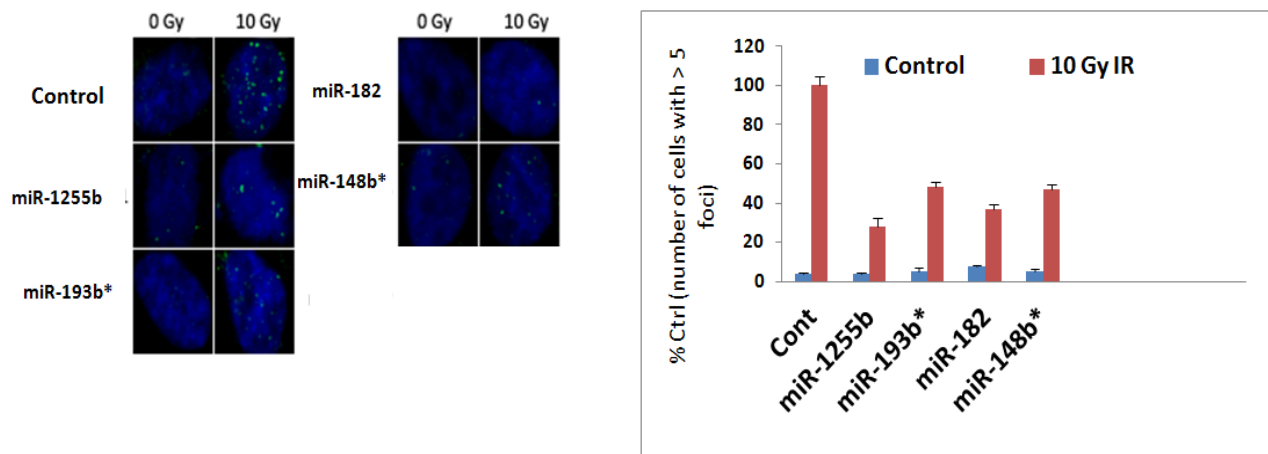

Figure 12. miRNA mimics for miR-1255b, miR-148b*, and miR-193b* and miR-182 inhibit HR repair in CCNE1 amplified OVCAR3 cells

2) These miRNAs synergize with platinum and PARP-inhibitors against CCNE1-amplified cell lines, that is expression of these miRNAs sensitizes CCNE1-amplified cells to platinum and PARP-inhibitors.

Another important goal of Aim 3, was to assess whether these miRNAs may synergize with platinum and PARPis in CCNE1-amplified cells.

To achieve this, luminescence based viability assay was performed in OVCAR3 and OVCAR4 ovarian cells. Cells were transfected with control miRNA, miRNA mimics for miR-1255b, miR-148b* and miR-193b* or BRCA1 siRNA (positive control). All cells were concomitantly treated with 1µM PARP inhibitor AZD2281 (Olaparib) and increasing concentrations of cisplatin for 5-6 days before ATP quantification.
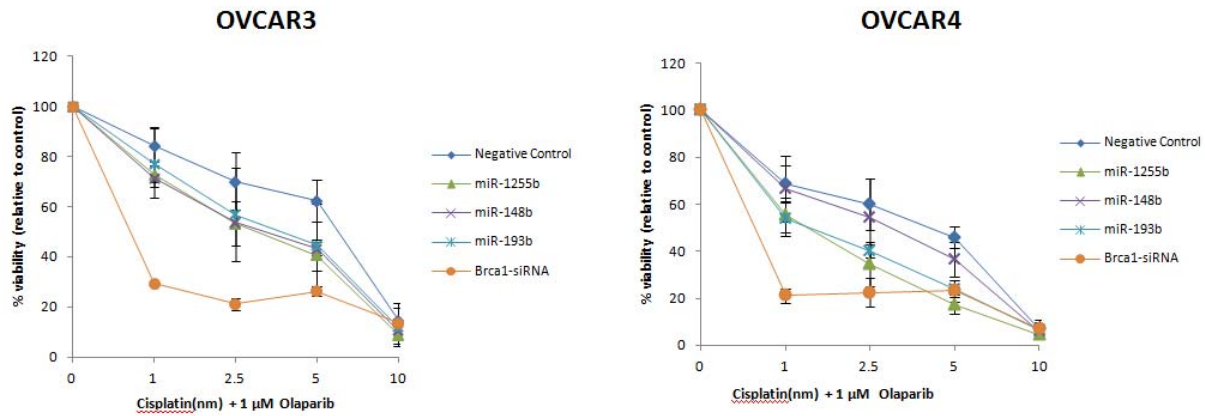

Figure 13. miRNA mimics for miR-1255b, miR-148b* and miR-193b* enhance cytotoxicity to cisplatin and olaparib.

As shown in Figure 13, miRNA mimics for miR-1255b, miR-148b* and miR-193b* enhanced sensitivity to cisplatin and olaparib, more than the negative control and less than the positive control BRCA1 siRNA. These findings, together with our finding that these miRNA mimics inhibit HR in CCNE1 amplified cells, support our hypothesis and suggest a novel strategy for targeting CCNE1 amplified tumors.

3) In vivo relevance of the miRNAs.

We generated OVCAR4 cells stably over-expressing these miRNAs from a chimeric construct and confirmed that these cells were hypersensitive to olaparib. Mice with xenografts of OVCAR4 cells expressing miRNAs or the scrambled control were treated with olaparib at a dose that typically does not impede tumor growth (Fig. 14, left panel). As anticipated the control tumors did not respond to olaparib but tumors expressing the miRNAs had a significant reduction in tumor
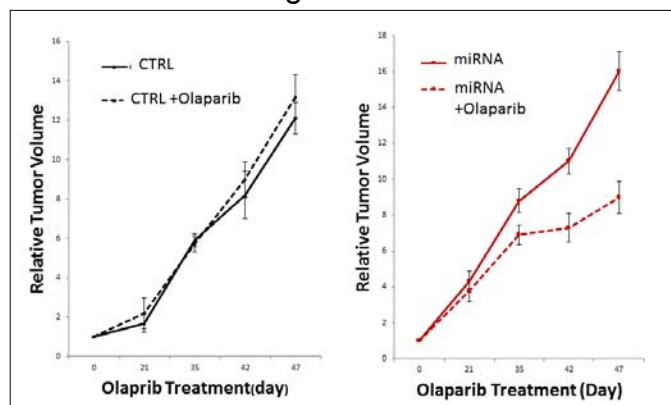
Figure 14. miRNA-1255b , miR-148b* and miR-193b* impact sensitivity to olaparib in orthotopic xenograft mouse models. Ovcar4 cells stably expressing control miRNA or miR-1255b-148b* were surgically implanted in nude mice and vehicle or PARP inhibitor treatment was initiated upon reaching tumor size of 200 mm^3 (n=20). Tumor volume was determined in 7 day-intervals and relative tumor volume was represented graphically, mean ± SD of all animals in each group.

volume (Fig. 14, right panel). The impact of olaparib on tumors expressing miRNAs was further confirmed by staining for caspase 3 activity (marker for apoptosis) and Ki67 (marker for proliferation). There was a clear increase in apoptotic activity and a decrease in proliferation in response to olaparib only in tumors derived from miRNA expressing cells (Fig. 15). These results strongly indicate that miR-1255b, miR-148b*, and miR-193b* sensitize CCNE- amplified ovarian cancer cell cells to PARP inhibitors both *in vitro* and *in vivo*, and there is potential for using them as therapeutic agents.
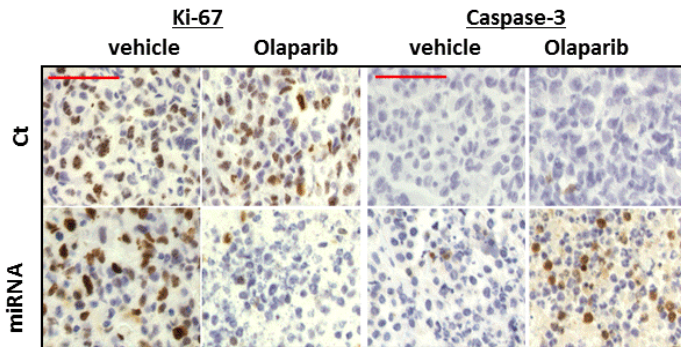
Figure 15. Tissues from control- or miRNA-expressing tumors with or without PARP inhibitor were stained for Ki-67 or caspase-3 to measure proliferation or apoptosis, respectively, in the tumors. Scale bar indicates 50 um.
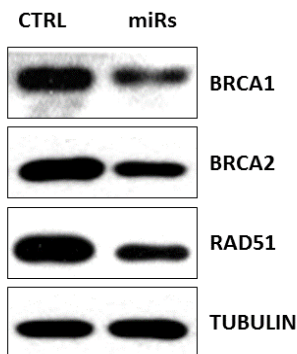
Figure 16. Tissues from control- or miRNA-expressing tumors were isolated and lysed in SDS-loading buffer. Immunoblot was conducted using the indicated antibodies.

In preliminary studies we had determined that that miRNAs, miR-1255b, miR-148b*, and miR-193b* target the DNA repair factors BRCA1, BRCA2 and RAD51. Next we investigated whether the targets of the miR-1255b, miR-148b*, and miR-193b* that were determined in preliminary studies remains unaltered in the xenografts. The tumors were retrieved from the animals and immunoblot conducted (Figure 16). Consistent with our previous results and the impact of the miRNAs on olaparib sensitivity, there is indeed an impact of the miRNAs on the levels of BRCA1, BRCA2 and RAD51.

4. Systemic identification and characterization of CCNE1-amplified ovarian cancer cell lines.

We obtained ~20 ovarian cell lines with different genotypes (such as BRCA1 mutant, BRCA2 mutant, others). First, we confirmed the genotype at DFCI cell profiling core facility, optimized growth conditions for these lines and then evaluated CCNE expression. CCNE expression was examined by immunoblot (Figure 17). Considering the variability in CCNE expression multiple experiments were performed with cells after
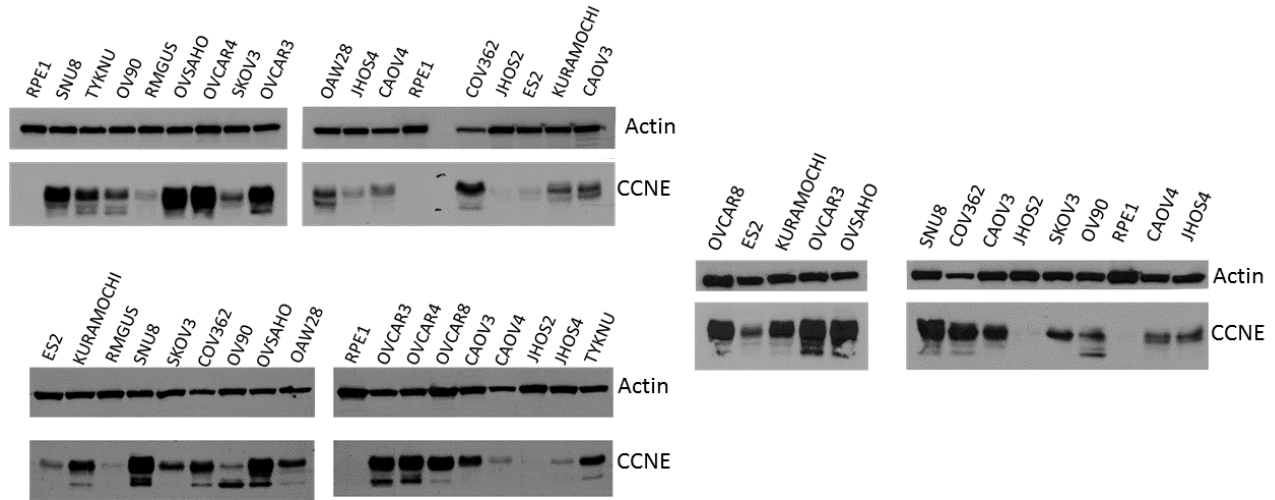
several passages. Independent experiments are shown.
Next we took the same cell lysates and proved for phosphorylated CHK1, a marker for replication stress.
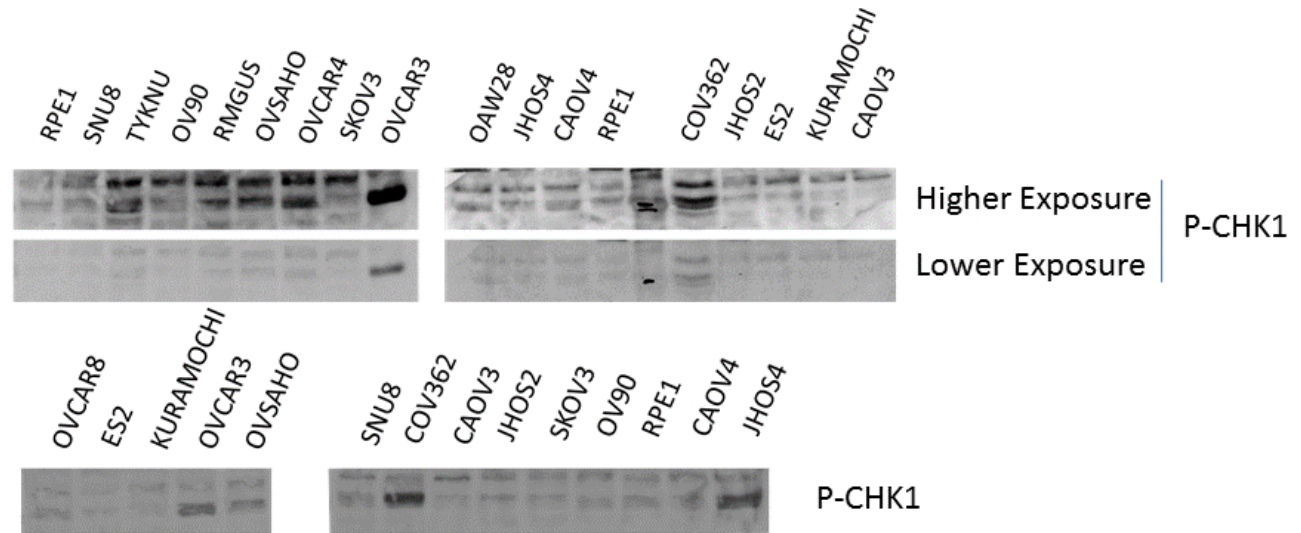
Figure 17. Cell lysates were probed by immunoblot for p-CHK1. Multiple independent experiments with the cell lines are shown

If indeed the CCNE1 expression is causing significantly enhanced proliferation than these cells may have a basal increase in pCHK1. The goal is to identify cell lines with the most pronounced replication stress.

Interestingly, COV362 the cell line with the consistently high pCHK1 levels is a BRCA1-mutant ovarian carcinoma cell lines. In a related project, we utilized these cells to

systematically characterize resistance to PARPis. Briefly, we employed a genome-scale CRISPR-Cas9 knockout library for identifying factors that would confer PARPi/platinum resistance to in Cov362. As expected, PARP1, 53BP1, and components of the Shieldin complex were uncovered in these screens. Importantly, we also found DYNLL1 and its transcriptional regulator ATMIN as top "hits", and we have shown that diminished DYNLL1 expression correlates with poor progression-free survival (PFS) of BRCA1-mutated ovarian cancer patients undergoing platinum-based chemotherapy. This study was recently published [14].

We have selected cell lines based on CCNE expression and inherent replication stress, and these are cells are being tested as xenografts, and their response to PARPis in vivo. In the future, we intend to express the miRNAs in the cells with the highest CCNE expression, which also grow well in animals, and test the impact on PARPis.

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

During this project Dr. Konstantinopoulos was promoted to Associate Professor of Medicine at Harvard Medical School and was named Director of Translational Research in the Gynecologic Oncology Program at Dana-Farber Cancer Institute. Furthermore, Dr. Chowdhury was promoted to Chief of Division of Radiation and Genome Stability in March 2017. He also became an Associate Editor of Oncogene in January 2017.

How were the results disseminated to communities of interest?

 "Nothing to Report."

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

"Nothing to Report."

4. **IMPACT:**

"Nothing to Report."

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

"Nothing to Report."

What was the impact on other disciplines?

"Nothing to Report."

What was the impact on technology transfer?

"Nothing to Report."

What was the impact on society beyond science and technology?

"Nothing to Report."

5. CHANGES/PROBLEMS:

"Nothing to Report."

Changes in approach and reasons for change

"Nothing to Report."

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

"Nothing to Report."

Changes that had a significant impact on expenditures

"Nothing to Report."

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

"Nothing to Report."

6. PRODUCTS:

Journal publications.

1. George E, Kim H, Krepler C, Wenz B, Makvandi M, Tanyi JL, Brown E, **Zhang R**, Brafford P, Jean S, Mach RH, Lu Y, Mills GB, Herlyn M, Morgan M, Zhang X, Soslow R, Drapkin R, Johnson N, Zheng Y, Cotsarelis G, Nathanson KL, Simpkins F. 2017. A patient-derived-xenograft platform to study BRCA-deficient ovarian cancers. JCI Insight 2:e89760. PMC5214535.

2. Kim H, George E, Ragland RL, Rafail S, **Zhang R**, Krepler C, Morgan MA, Herlyn M, Brown EJ, Simpkins F. 2016. Targeting the ATR/CHK1 Axis with PARP Inhibition Results in Tumor Regression in BRCA-Mutant Ovarian Cancer Models. Clin Cancer Res. PMID: 27993965.

3. Stephen TL, Payne KK, Chaurio RA, Allegrezza MJ, Zhu H, Perez-Sanz J, Perales-Puchalt A, Nguyen JM, Vara-Ailor AE, Eruslanov EB, Borowsky ME, **Zhang R**, Laufer TM, Conejo-Garcia JR. 2017. SATB1 Expression Governs Epigenetic Repression of PD-1 in Tumor-Reactive T Cells. Immunity 46:51-64. PMC5336605.

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5. Yokoyama Y, Zhu H, Lee JH, Kossenkov AV, Wu SY, Wickramasinghe JM, Yin X, Palozola KC, Gardini A, Showe LC, Zaret KS, Liu Q, Speicher D, Conejo-Garcia JR, Bradner JE, Zhang Z, Sood AK, Ordog T, Bitler BG, **Zhang R**. 2016. BET Inhibitors Suppress ALDH Activity by Targeting ALDH1A1 Super-Enhancer in Ovarian Cancer. Cancer Res 76:6320-30. PMC5117661.

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7. Bitler BG, Wu S, Park PH, Hai Y, Aird KM, Wang Y, Zhai Y, Kossenkov AV, Vara-Ailor A, Rauscher Iii FJ, Zou W, Speicher DW, Huntsman DG, Conejo-Garcia JR, Cho KR, Christianson DW, **Zhang R**. 2017. ARID1A-mutated ovarian cancers depend on HDAC6 activity. Nat Cell Biol 19:962-973. PMID: 28737768. PMC5541905.

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9. Zeng M, Kwiatkowski NP, Zhang T, Nabet B, Xu M, Liang Y, Quan C, Wang J, Hao M, Palakurthi S, Zhou S, Zeng Q, Kirschmeier PT, Meghani K, Leggett AL, Qi J, Shapiro GI, Liu JF, Matulonis UA, Lin CY, **Konstantinopoulos PA***, Gray NS*. Targeting MYC dependency in ovarian cancer through inhibition of CDK7 and CDK12/13. Elife. 2018 Nov 13;7. pii: e39030. doi: 10.7554/eLife.39030. PubMed PMID: 30422115; PubMed Central PMCID: PMC6251623.

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*Joint Corresponding Author

Books or other non-periodical, one-time publications.

"Nothing to Report."

Other publications, conference papers, and presentations.

"Nothing to Report."

- **Website(s) or other Internet site(s)**

"Nothing to Report."

- **Technologies or techniques**

"Nothing to Report."

- **Inventions, patent applications, and/or licenses**

"Nothing to Report."

- **Other Products**

"Nothing to Report."

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:
What individuals have worked on the project?

Name:	*Panagiotis Konstantinopoulos*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Konstantinopoulos has performed work related to the Aim 1 of this project and supervised the overall study.
Funding Support:	Stand up to Cancer,

Name:	Pascal Drane (Chowdhury)
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6 calendar months
Contribution to Project:	Dr. Drane worked together with Dr. Zheng and Ms. Moore in optimizing conditions for growth of ovarian cancer lines and performing the experiments characterizing these lines. did the PARPi sensitivity and foci forming assays
Funding Support:	DoD, NIH

Name:	Jessica Moore (Chowdhury)
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6 calendar months
Contribution to Project:	Ms. Moore assisted Drs. Drane and Zheng in optimizing conditions for growth of ovarian cancer cells.
Funding Support:	DoD, The Honorable Tina Brozman Foundation

Name:	*Dipanjan Chowdhury*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.6 calendar months
Contribution to Project:	Dr. Chowdhury has performed work related to the Aim 2 of this project.
Funding Support:	DoD, The Honorable Tina Brozman Foundation NIH AI101894, NIH CA142698, NIH CA208244, LLS.

Name:	Walker Fuchs (Chowdhury)
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3.48 calendar months
Contribution to Project:	*Mr. Fuchs has performed work in the area of combined error-control and constrained coding*

Funding Support:	DoD

Name:	Xiao-Feng Zheng (Chowdhury)
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.2 calendar months
Contribution to Project:	Dr. Zheng worked in optimizing conditions for growth of ovarian cancer lines and performing the experiments characterizing these lines.
Funding Support:	DoD, NIH CA208244.
Name:	*Rugang Zhang*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Supervised the study on specific aim 3.
Funding Support:	This award

Name:	*Sergey Karakashev (Zhang)*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	*5*
Contribution to Project:	Performed the study.
Funding Support:	This award

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

"Nothing to Report."

What other organizations were involved as partners?

"Nothing to Report."

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES: None

Cited References for this report:

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14.   He, Y.J., et al., *DYNLL1 binds to MRE11 to limit DNA end resection in BRCA1-deficient cells.* Nature, 2018. **563**(7732): p. 522-526.