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CONTRACTING ORGANIZATION: **University of Pittsburgh**

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14. ABSTRACT:
This Research Plan is testing a readily-deployed novel drug strategy for treating TNBC, where the inhibition of Rad51-mediated DNA repair by electrophilic nitroalkenes renders TNBC cells more sensitive to PARP inhibition and TNBC cell killing. OA-NO_2 and NFA-8 are nitroalkenes, 7-nitro-nonadec-7-enoic acid and 10-nitro-octadec-9-enoic acid, respectively. We have now devised an even more pharmacologically efficacious nitroalkene that shares the same active nitroalkene moiety with OA-NO_2 and NFA-8, dimethyl-4-nitro-oct-4-enoiate (CP-1). New data indicates that CP-1 outperforms both OA-NO_2 and NFA-8, respectively. CP-1 shows not only enhanced cell killing of TNBC cells as single drugs as well as combination therapy, but also displays cell protective effects in benign breast epithelial cells that are treated with PARP inhibitors or ionizing radiation. Further, preliminary data also show that smaller alkynyl (R-C≡CH) terminus on OA-NO_2 facilitates secondary "click" reactions with azido-substituted affinity tags that facilitate protein pull-down as well as HPLC-MS/MS-based detection. Preliminary studies support click chemistry-based Rad51 and β-actin pulldown through alkynyl-tagged NFAs from cell lysate.

15. SUBJECT TERMS
Nitroalkenes, triple negative breast cancer, homologous recombination directed DNA repair, PARP, RAD51

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1. INTRODUCTION:

This Research Plan will test a readily-deployed novel drug strategy for treating TNBC, where the inhibition of Rad51-mediated DNA repair by electrophilic NFA renders TNBC cells more sensitive to PARP inhibition and TNBC cell killing. 15% of all breast cancers are triple negative, being devoid of the three receptors that classify and define treatment strategies for most mammary cancers: estrogen receptor (ER), progesterone receptor (PR) and ERB2 (also known as HER2). Because of this, no targeted therapies are available for TNBC patients. This poses a destitute situation, as TNBC is an aggressive cancer that disproportionally affects younger women and those having African origins. 15-20% of TNBC patients also carry a germline *BRCA1* or *BRCA2* mutation, resulting in defects in homologous-directed DNA repair (HDR). Two open-label Phase 3 clinical trials have now signified the advantage of PARPi monotherapy compared to chemotherapy for germline *BRCA*-mutant TNBC patients. In the OlympIAD trial, progression-free survival was prolonged by 2.8 mo. and in the EMBRACA trial by 3 mo. These findings are promising and significant, as they reinforce the concept that genetic defects in HDR pave a path to PARPi cancer cell killing, by inhibiting single strand DNA repair. This in turn underscores the significance of our Research Plan. We propose to induce HDR-deficiency through Rad51 inhibition, which then in turn will amplify PARPi efficacy. To accomplish this, we have identified a unique reactivity of a novel class of Rad51 inhibitors, nitro alkenes, which are well tolerated and readily testable as new drug candidates in humans: nitro alkene derivatives are endogenously present as nitrated fatty acids in humans and their synthetic homologs have been de-risked by preclinical toxicology and 5 Phase 1 safety evaluations of IV and the oral nitro alkene OA-NO_2 in healthy and obese volunteers (http://www.complexarx.com), as well as ongoing Phase trials in renal and cardiopulmonary disease patients, presenting a promising new opportunity for treating the drug-resistant TNBC breast cancer phenotype.

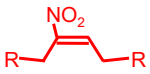

Fig.1 nitro alkene

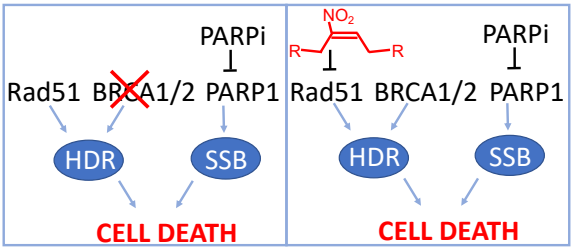

Fig. 2. Chemical induction mimicking loss of BRCA1/2 through targeting Rad51 with OA-NO_2 to induce cell death in TNBC.

2. KEYWORDS:

Triple negative breast cancer, PARP inhibition, DNA-double strand repair, Homologous directed DNA repair deficiency, RAD51, nitro fatty acid, nitroalkene

3. ACCOMPLISHMENTS:

- **MAJOR GOALS:**

Aim 1: Examine if NFA-dependent inhibition of HDR is exclusively mediated by Rad51 Cys319 thiol adduction.

Major Task 1/Neumann: Generation of TNBC CRISPR cell lines expressing Rad51 mutants (MDA-MB-231, BT49 and Hs578T from ATCC TNBC cell line panel). *Completion 65%*

Major Task 2/Freeman: NFA-8 synthesis. *Completion 100%*

Major Task 3/Neumann: Use TNBC CRISPR cell lines and compare responses of OA-NO_2 or NFA-8 plus olaparib. *Completion 0%*

Major Task 4/Neumann: Generate Rad51 recombinant proteins (WT and Cys319Ser) and test effects of NFAs on Rad51 DNA binding, oligomerization, presynaptic filament formation and ATPase activity. *Completion 30%*

Major Task 5/Freeman: Determination of OA-NO_2 protein adduction sites in Rad51 and other HDR proteins. *Completion 50%*

Aim 2: Compare the structure-function relationships of OA-NO_2 with NFA-8 in a panel of 10 TNBC cell lines for HDR-inhibition and lethality by determining combination and drug reduction indices.

Major Task 6/Neumann: Determine proliferation and viability of 10 TNBC cell lines (BT549, BT20, HCC1500, HCC1937, HCC1187, MDA-MB-231, MDA-MB-436, SUM149PT, SUM159PT and HS578T) and 2 immortalized breast epithelial cell lines (MCF-10A, MCF-12A) in response OA-NO_2, NFA-8 and olaparib. *Completion 35%*

Major Task 7/Neumann: Calculation of combination and drug reduction indices (CI and DRI) using CalcuSyn software. *Completion 50%*

Major Task 8/Neumann: Compare OA-NO_2 and NFA-8 responses in TNBC cell lines and benign breast epithelial in DNA DSB repair. *Completion 50%*

Aim 3: Utilize human TNBC patient-derived xenografts (PDXs) and a mutant p53 breast cancer mouse model to evaluate drug synergism of NFAs in combination with PARP inhibition.

Major Task 9/Neumann: Apply for IACUC approval and ACURO approval. *Completion 100%*

Major Task 10/Neumann and Freeman: Human *ex vivo* explants. *Completion 0%*

Major Task 11/Neumann and Freeman: Expand PDXs in Balb/c mice/immunogenic mouse model. *Completion 0%*

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

To Major Task 1/Neumann: Generation of TNBC CRISPR cell lines expressing Rad51 mutants (MDA-MB-231, BT49 and Hs578T from ATCC TNBC cell line panel).

CRISPR mutant clones have been generated for MDA-MB-2131 and MCF-10A cells. We are currently expanding the clones, which then will be sequenced to identify successfully target clones.

Major Task 2/Freeman: NFA-8 synthesis:

OA-NO_2 and NFA-8 are nitroalkenes, 7-nitro-nonadec-7-enoic acid and 10-nitro-octadec-9-enoic acid, respectively. We have now devised an even more pharmacologically efficacious nitroalkene that shares the same active nitroalkene moiety with OA-NO_2 and NFA-8, dimethyl-4-nitro-oct-4-enoiate (CP-1). New data indicates that CP-1 outperforms both OA-NO_2 and NFA-8, respectively (data shown below). 25 gm of CP-1 have been synthesized and is available for both in vitro and in vivo studies.

Major Task 4/Neumann: Generate Rad51 recombinant proteins (Wt and Cys319Ser) and test effects of NFAs on Rad51 DNA binding, oligomerization, presynaptic filament formation and ATPase activity.

We have tested Wt and recombinant Rad51-Cys319Ser reaction with OA-NO_2 and found that OA-NO_2 has the ability to specifically disrupt Rad51

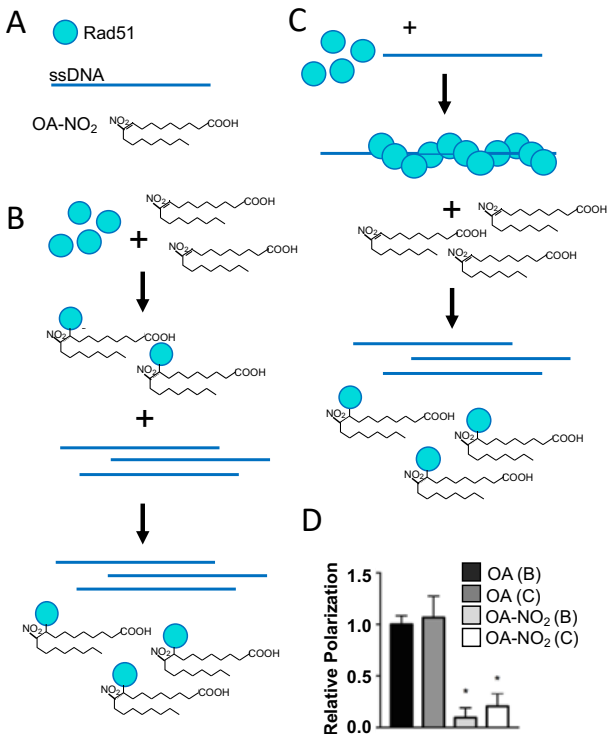

Fig. 3. A. DNA binding was measured using fluorescence polarization (FP) on a Tecan Spark 20M (ex/em 480 nm/535 nm). **B.** Purified Rad51, ATP and 5 μM OA or 5 μM OA-NO_2 was first incubated for one hour before Alexa Fluor 488 conjugated single strand (ss) DNA was added. Fluorescence polarization was quantified and normalized to a control lacking ATP. **C.** Purified Rad51, ATP was incubated for one hour with Alexa Fluor 488 conjugated single strand (ss) DNA. Then, 5 μM OA or 5 μM OA-NO_2 were incubated for two hours Fluorescence polarization was quantified and normalized to a control lacking ATP. **D**. Quantification. Average + SEM, n = 3.

binding from DNA. This was probed by quantifying changes in fluorescence polarization of an Alexa Fluor 488 conjugated single-stranded oligonucleotide *in vitro*. In the presence of ATP, Rad51 protein binding to Alexa Fluor 488 conjugated ssDNA will increase the polarization of light emitted by the fluorescent substrate (**Fig. 3A**) (4). OA-NO_2, but not OA, decreased the relative polarization of Rad51 in the presence of ATP and DNA (**Fig. 3D**). This inhibition of polarization increase was observed both a) when OA-NO_2 was pre-incubated with Rad51 and then mixed with DNA (**Fig. 3B**), and b) when Rad51 was already associated with DNA, and then OA-NO_2 or OA was added (**Fig. 3C**). This suggests OA-NO_2 robustly abrogates Rad51-DNA interaction by a) not only preventing Rad51 from DNA binding, but also disrupting already assembled Rad51 oligomers. Control experiments found that OA and OA-NO_2 did not cause non-specific effects such as fluorophore quenching-induced decreases in fluorescence polarization (not shown). This suggests that OA-NO_2 can access C319 of Rad51 monomers in a filament and dissociate Rad51 protein from ssDNA *in vitro*.

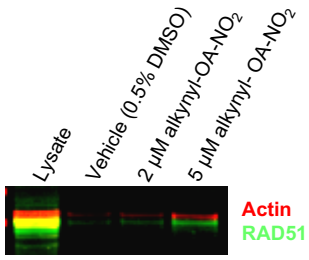

Fig. 4. Click Chemistry-linked affinity capture: In UWB1.289 cells Rad51 and b-actin cysteines were adducted by alkynyl OA-NO_2 and purified via biotin/streptavidin beads.

As proposed, we are currently generating Rad51 mutant proteins (Rad51 Cys319Ser) with our collaborator Dr. Patrick Sung and will soon compare Rad51 WT with mutant protein in the assays listed and compare OA-NO_2 with CP-1 in these assays.

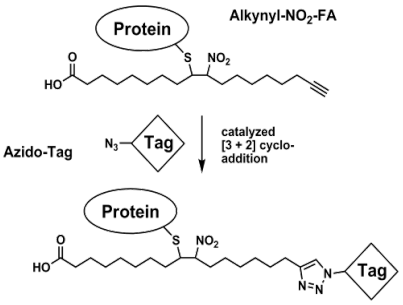

Fig. 5. Click chemistry strategy for detecting protein-adducted NFA. The alkynyl substituent reacts with diazo-biotin and is purified via streptavidin (beads).

Major Task 5/Freeman: Determination of OA-NO_2 protein adduction sites in Rad51 and other HDR proteins.

We have previously used biotinylated electrophilic lipids NFA for affinity-based identification of NFA-protein adduct formation specific proteins, organelles and cells (45, 46). Biotin is bulky (244 g/mol) compared with various NFA species (300-350 g/mol). Thus, integrating a smaller alkynyl (R-C≡CH) terminus on OA-NO_2 facilitates secondary "click" reactions with azido-substituted affinity tags that facilitate protein pull-down as well as HPLC-MS/MS-based detection. Preliminary studies support click chemistry-based Rad51 and β-actin pulldown through alkynyl-tagged NFAs from cell lysate (**Figs. 4 and 5**). This allows specific identification of this and other potential NFA targets in SSA and Alt-EJ-DNA repair pathways. This innovative approach was designed to reveal the degree of specificity of OA-NO_2 Rad51 targeting and lays the foundation for better characterizing new NFA regioisomers and their molecular targets.

Our next step is to identify OA-NO_2 alkynyl-adducted cysteines in cytoplasmic and nuclear cell fractionations using mass spectrometry. U2OS cells (+/-) irradiation will be analyzed in triplicates. We will focus on identifying proteins involved in DNA DSB repair.

Major Task 6/Neumann: Determine proliferation and viability of 10 TNBC cell lines (BT549, BT20, HCC1500, HCC1937, HCC1187, MDA-MB-231, MDA-MB-436, SUM149PT, SUM159PT and HS578T) and 2 immortalized breast epithelial cell lines (MCF-10A, MCF-12A) in response OA-NO_2, NFA-8 and olaparib.

We have compared OA-NO_2 with the newly identified nitroalkene CP-1 in proliferation and apoptosis assays. MCF10A and MDA-MB-231 cells were respectively infected with IncuCyte® NucLight Red Lentivirus in order to produce red nuclei for the apoptosis IncuCyte assay. Four wells of a 96-well plate were plated with cells at appropriate density the day prior. One well was treated with the reagent with a MOI of 1 and the

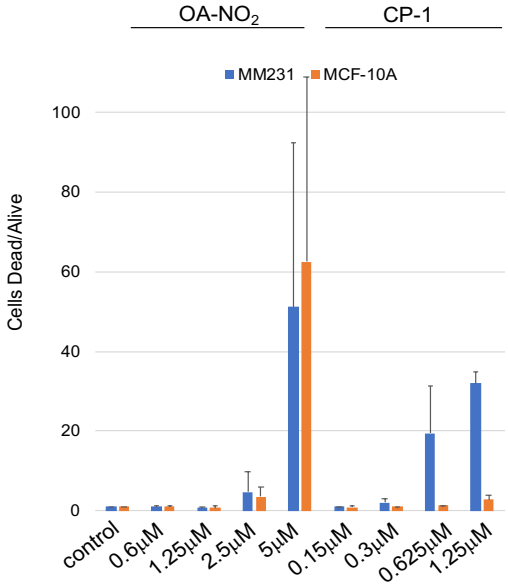

Fig. 6. MDA-MB-231 (MM231, blue) and MCF-10 (red) cells were analyzed for toxicity by OA-NO_2 and CP-1. Cells were treated on day 1 and live as well as dead cells were analyzed on day 3. n=2

other well treated with a reagent with MOI of 3. After the infected cells reached appropriate confluency, they were transferred to a 6-well plate to further proliferate. Cells with red nuclei were selected using fluorescence activated cell sorting (FACS).

Sorted cells (MDA-MB-231 and MCF-10A) were seeded at a density of 2 x 10^3/well on a clear 96-well plate. The next day, cells were incubated with apoptotic markers Cytotox Green (Cat. No. 4633), Annexin V (Cat. No. 4642) and the PARPi olaparib at desired concentrations. Wells were then co-treated with OA-NO_2 or CP1 and then analyzed in the IncuCyte over a time course of 3 days. Nuclei (live cell marker) and apoptotic markers were quantified and the ratio of apoptotic to live cells at the end of day 3 is shown in **Fig. 6.** Notably, OA-NO_2 dosing increased cell death in both, MDA-MB-231 cells and MCF-10A cells, while CP-1 only induced toxicity to the TNBC cell line MDA-MB-231 but not the benign cell line MCF-10A.

Utilizing the same assay, both cell lines were further analyzed for responses to both, OA-NO_2 or CP-1 and olaparib. As show in **Fig. 7** in contrast to the highest dose of OA-NO_2, CP-1 at both 0.625 μM and 1.25μM shows a protective effect in MCF10A cells co-treated with olaparib. These data indicate that CP-1 shows not only higher in vitro tumor cell killing potency compared to OA-NO_2 but also may provide a protective effect towards benign MCF-10A cells when treated with olaparib. Further replicates of these experiment are in progress.

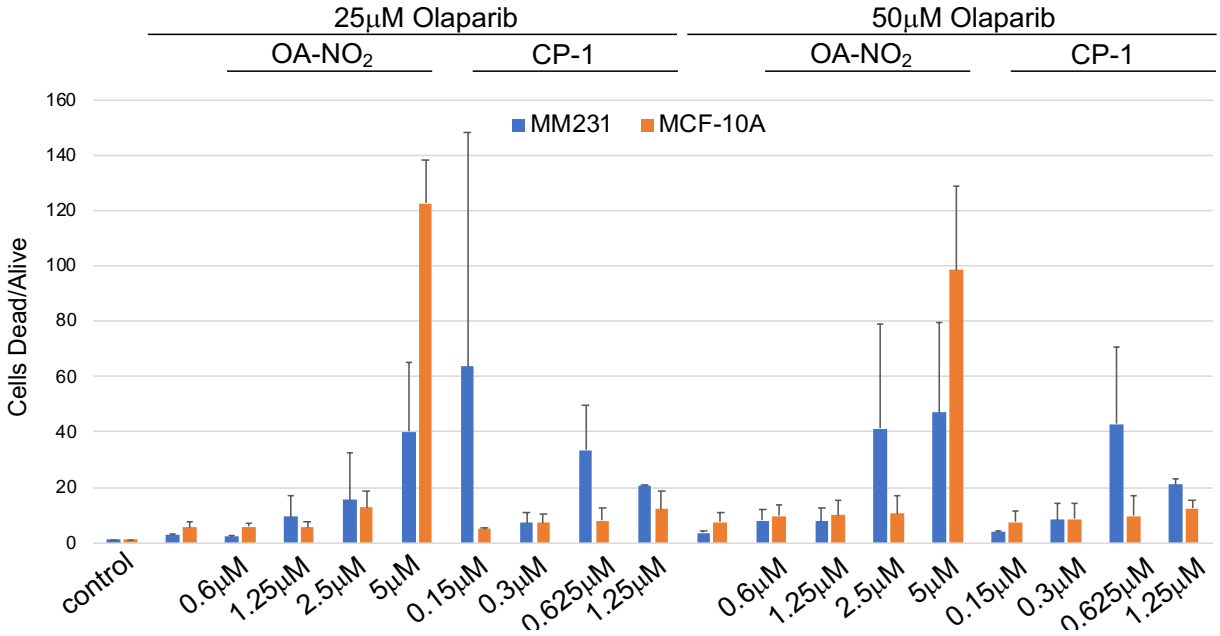

Fig. 7. MDA-MB-231 (MM231) and MCF-10 cells were analyzed for toxicity induced by OA-NO_2 and CP-1 in combination with two different doses of olaparib (ola). Cells were treated on day 1 and live as well as dead cells were analyzed on day 3. n=2

OA-NO_2 and CP-1 potencies were also compared by clonogenic analysis in 6 well plates. 500 cells/well were plated and treated the following days with OA-NO_2 or CP-1. As shown in **Fig. 8**, 0.5μM and 1μM CP-1 significantly decreased clonogenic outgrowth in MDA-MB-231 cells but not in MCF-10A cells.

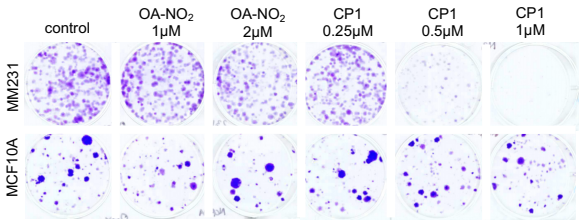

Fig. 8. Comparison of OA-NO_2 and CP-1 for clonogenic survival

To test if CP-1 has a protective effect on MCF-10A cells in response to ionizing radiation (IR), MDA-MB-231 cells as well as MCF-10A cells were examined again for clonogenic survival in 6 well plates. 500 cells/well were plated, then irradiated and subsequently treated with OA-NO_2 or CP-1 12 hr later. Again, CP-1 protects MCF-10A cells from IR toxicity as MCF-10A cells treated with IR only show lower number of colonies than cells treated with IR and CP-1 (**Fig. 9**). This is especially evident in cells treated with 4Gy. In MDA-MB-231 cells, co-treatment of CP-1 with IR shows lower colony numbers compared to MDA-MB-231 cells treated with IR and OA-NO_2. These data emphasize that CP-

1 protects benign cells from DNA damage induced by PARPi or IR and augments both DNA damage and cell death in TNBC cells.

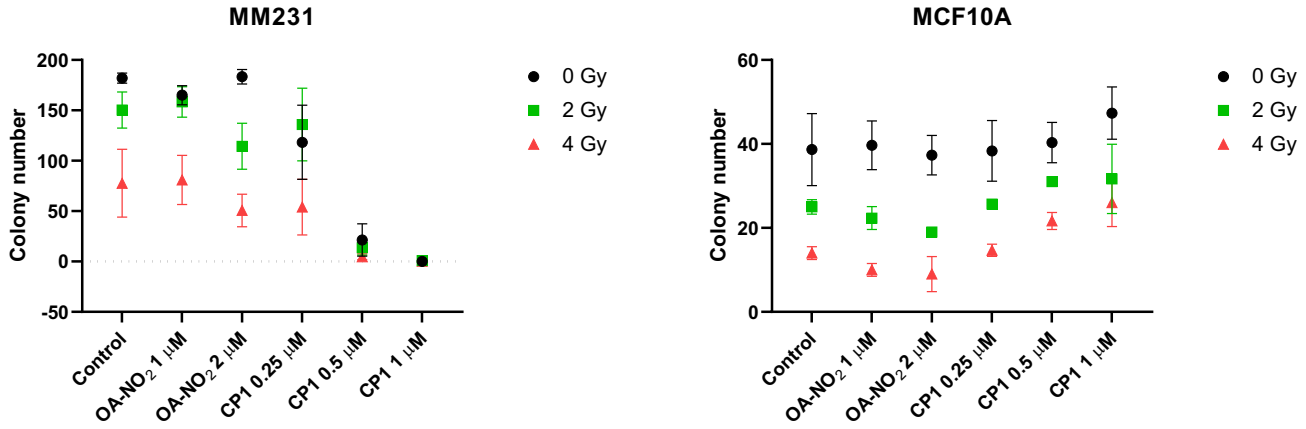

Fig. 9, CP-1 protects the benign breast epithelial cell line MCF-10A from toxic effects of IR while it potentiates IR effects in the TNBC cell line MDA-MB-231; n=3

Major Task 7/Neumann: Calculation of combination and drug reduction indices (CI and DRI) using CalcuSyn software.

MDA-MB-231 cells were analyzed for drug synergism between OA-NO_2 or NFA-8 and the PARP inhibitors olaparib and talazoparib. For this, relative cell numbers were first compared by measuring the luminescent signal generated by ATP using the CellTiter-Glo (Promega) assay. 5 x 10^3 MBA-MD-231 cells/well were plated in a 96-well plate and treated with PARPi at the indicated concentrations for 72 h in the presence or absence of OA-NO_2 or the control oleic acid (OA) and replenished every 24 h. Compusyn software was used to calculate the combination index values and synergy for PARP inhibitors in combination with OA-NO_2. As shown in **Fig. 10** co-treatment of OA-NO_2 with talazoparib, showed higher drug synergism compared to OA-NO and olaparib.

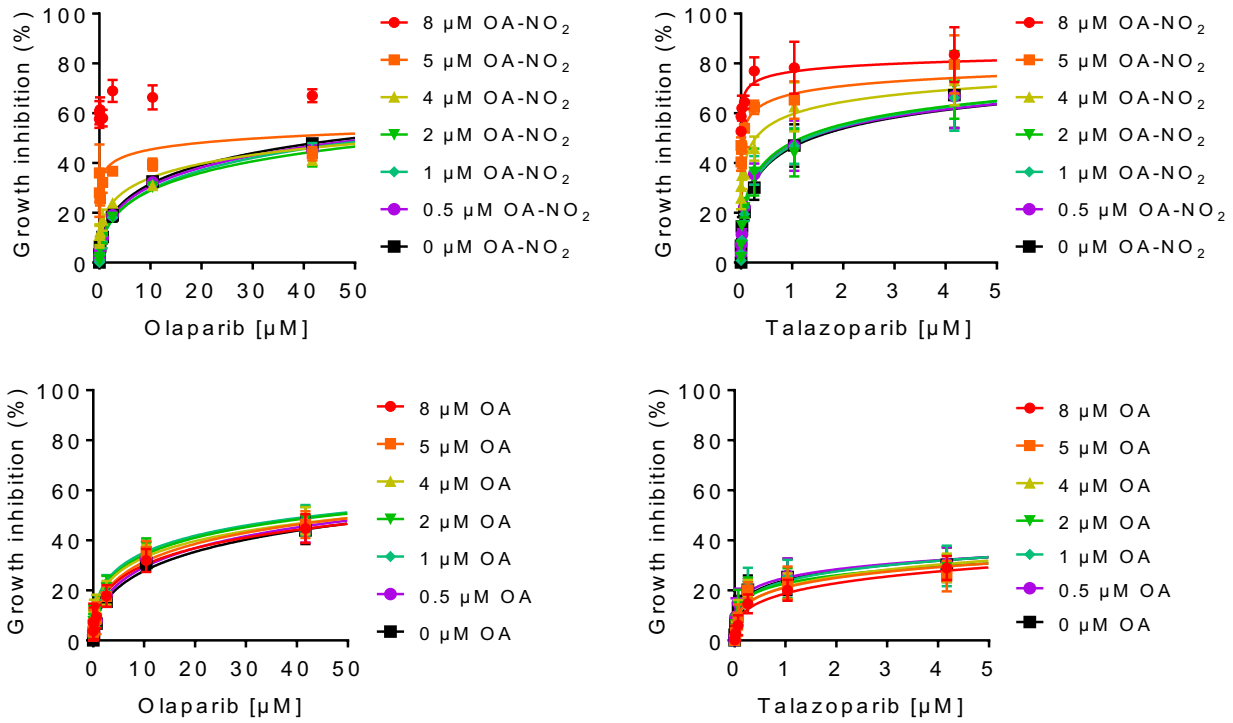

Fig. 10. Co-treatment of MDA-MB-231 cells with OA-NO_2 NFA-8 and the PARP inhibitors olaparib and talazoparib; n=3

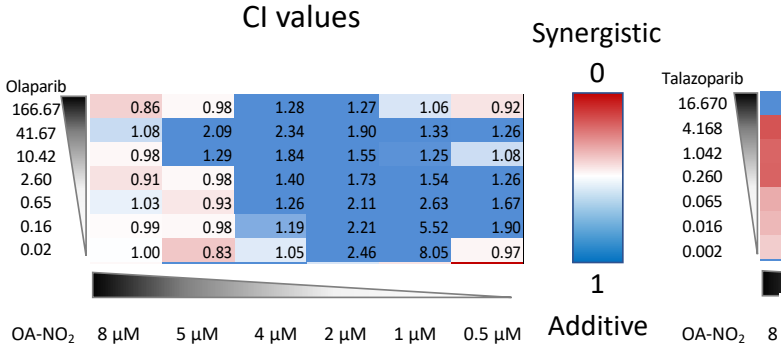

Fig. 11. Drug synergism between OA-NO_2 and the PARP inhibitors olapar

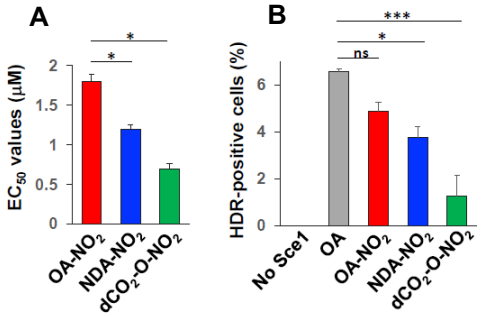

Fig. 14. NFA-8 (7-NDA-NO_2) and CP-1 (diCO_2-O-NO_2) show (A) lower EC_{50} than OA-NO_2 for the inhibition of MDA-MB-231 TNBC cell growth and (B) the greatest extent of inhibition of HDR in U2OS GFP-reporter constructs. Data from 3 independent exps, mean+SEM; n=3.

X-ray modeling studies of the Rad51-Cys319 region revealed that, as opposed to OA-NO_2 (shown), if one were to synthesize a nitro-fatty acid that has the nitroalkene substituent closer to the carboxylate terminus, there would be an enhancement of hydrogen bonding between the carboxylate and Rad51-Glu322. This also predicted stronger hydrophobic interactions with Pro318, increased Cys319 alkylation and greater inhibition of HDR and tumor cell growth and survival. Thus, 7-NO_2-nonadecenoic acid (NDA-NO_2/NFA-8) was synthesized for comparison with OA-NO_2. Next, utilizing the same assay, OA-NO_2 and NFA-8 were compared and analyzed for drug synergism with talazoparib. As shown in **Fig. 11**, syngergistic growth inhibition is higher in MDA-MB-231 cells co-treated with talazoparib comapred to OA-NO_2. <u>These data suggest that NFA-8 is more efficacious in killing TNBC cells syngergistically when combined wirth talazoparib compared to OA-NO_2</u>

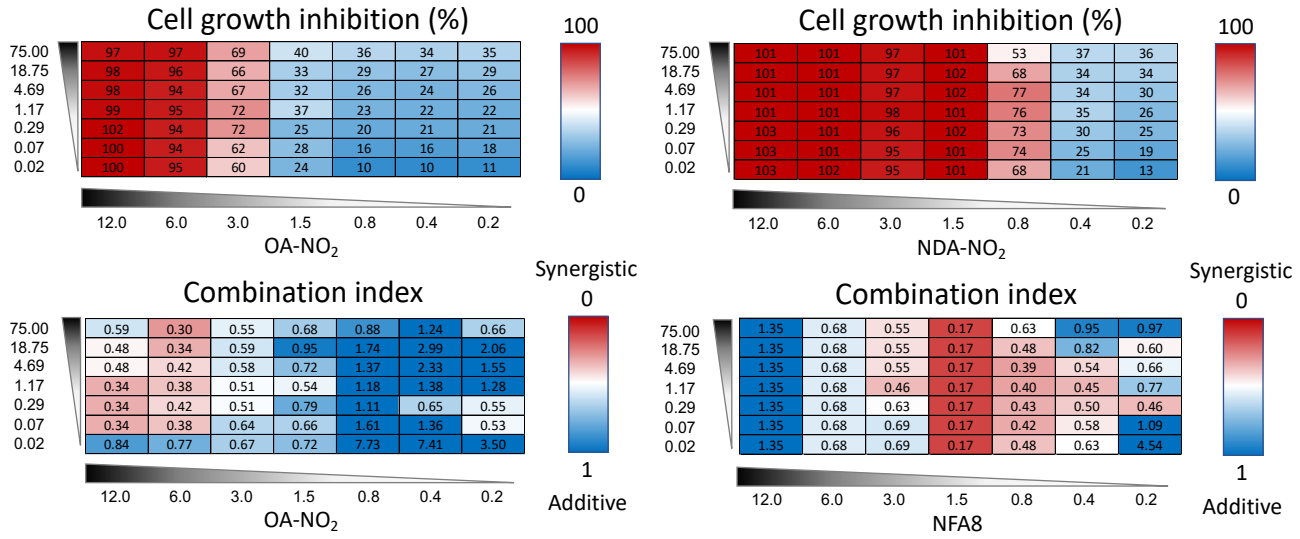

Fig. 12. Comparison of drug synergism between OA-NO_2 , NFA-8 and the PARP inhibitors talazoparib. n=3

Then, OA-NO_2, NFA-8 and CP-1 were compared for effects on cell proliferation by using the CellTiter-Glo assay. These results suggested that diCO_2-O-NO_2 (CP-1) had a 63% lower EC50 compared to OA-NO_2, and 7-NO_2-nonadecenoic acid (NFA-8) an about 30% lower EC50 compared to OA-NO_2. This suggest that CP-1 is the more potent drug candidate out of all nitroalkenes tested (**Fig. 13) and ongoing are drug synergism studies with PARPi and CP-1**.

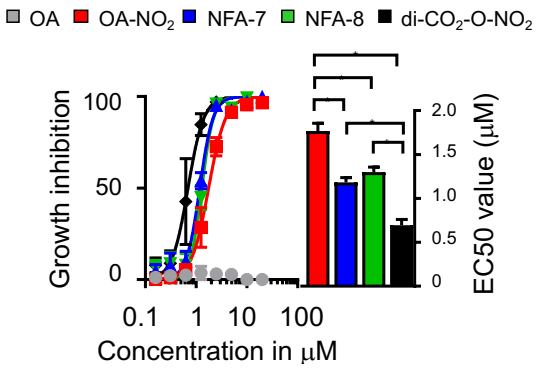

Fig. 13. Comparison of nitroalkenes for EC50 in MDA-MB-231 cells; n=3

Major Task 8/Neumann: Compare OA-NO_2 and NFA-8 responses in TNBC cell lines and benign breast epithelial in DNA DSB repair.

Utilizing the DR-GFP assay for HDR (33), both NFA-8 and CP-1 displayed better inhibition of HDR than OA-NO_2, as predicted, whereas CP-1 displayed the lowest EC50 as well as inhibition of HDR (**Fig. 14)**. The nitroalkene diCO_2-O-NO_2 is unique because as the diethylated prodrug it will be readily absorbed and, after de-esterification, is expected to avidly be transported into cells by members of the Organic Anion Transporter Polypeptide (OATP) superfamily. Appreciating that an array of OATPs are highly over-expressed in human tumor cells, including breast, liver, colon, pancreatic and ovarian cancers (57, 58), it is viewed that diCO_2-O-NO_2 will display superior half-life, tumor cell accessibility and Rad51 inhibition.

- **What opportunities for training and professional development has the project provided?**

In the process of conducting experimental endeavors, participating students and fellows have been trained in novel organic chemistry, cell/molecular biology and pharmacokinetics-related experimental methodologies, approaches to statistical analysis and data presentation.

- **How were the results disseminated to communities of interest?**

However, we will communicate our findings to bcRAN (breast cancer research advocacy network) at the coming bcRAN bootcamp in November 2020, where breast cancer survivor will come together in Pittsburgh at the Magee Womens Research Institute, which is affiliated with UPMC. In addition, we will present data at the Great Lakes Breast Cancer Symposium in October 2020 and at a Gordon Research Conference in July, 2020..

- **What do you plan to do during the next reporting period to accomplish the goals?**

To Major Task 1/Neumann: Generation of TNBC CRISPR cell lines expressing Rad51 mutants (MDA-MB-231, BT49 and Hs578T from ATCC TNBC cell line panel). We are currently expanding the clones, which then will be sequenced to identify successfully target clones.

To Major Task 3/Neumann: Once CRISPR clones are established, cell lines will be compared for responses of OA-NO_2, CP-1 plus olaparib.

To Major Task 4/Neumann: Generate Rad51 recombinant proteins (Wt and Cys319Ser) and test effects of NFAs on Rad51 DNA binding, oligomerization, presynaptic filament formation and ATPase activity. As proposed, we are currently generating Rad51 mutant proteins (Rad51 Cys319Ser) with our collaborator Dr. Patrick Sung and will soon compare Rad51 WT with mutant protein in the assays listed. OA-NO2 actions will be compared to CP-1 in these assays.

To Major Task 5/Freeman: Determination of OA-NO_2 protein adduction sites in Rad51 and other HDR proteins. Our next step is to identify OA-NO_2 alkynyl-adducted cysteines in cytoplasmic and nuclear cell fractionations using mass spectrometry. Following acquisition of this information, more selective ion monitoring strategies can be utilized for both the Rad51 Cys319-containing peptide and other OA-NO_2-targeted DNA repair proteins to define CP-1 adduction sites that are anticipated to be similar. U2OS cells (+/-) irradiation will be analyzed in triplicates. We will place primary focus on identifying proteins involved in DNA DSB repair.

Major Task 6/Neumann: Determine proliferation and viability of 10 TNBC cell lines (BT549, BT20, HCC1500, HCC1937, HCC1187, MDA-MB-231, MDA-MB-436, SUM149PT, SUM159PT and HS578T) and 2 immortalized breast epithelial cell lines (MCF-10A, MCF-12A) in response OA-NO_2, NFA-8, CP-1 and olaparib. Expand data and test other cell lines as shown in Figs. 6-13 for proliferation and viability, so that combination indicis can be determined as proposed in Major Task 7.

Major Task 8/Neumann: Compare OA-NO_2 and NFA-8 responses in TNBC cell lines and benign breast epithelial in DNA DSB repair as proposed.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

   - **What was the impact on the development of the principal discipline(s) of the project?** We are well along the pathway to discovery of a novel inhibitor of homologous recombination that a)

has already been shown from Phase 1 and 2 trials in renal and cardiopulmonary disease patients to be safe in humans, b) we have improved on the potency and pharmacokinetics of NFA-8 and OA-NO_2 (7-nitro-nonadec-7-enoic acid and 10-nitro-octadec-9-enoic acid, respectively) by devising an even more pharmacologically efficacious nitroalkene that shares the same active nitroalkene moiety with OA-NO_2 and NFA-8 (dimethyl-4-nitro-oct-4-enoiate, CP-1).

- **What was the impact on other disciplines?** In addition, the potential discovery of a transformative strategy in cancer therapeutics, we are advancing basic understanding of cell signaling, pharmacology, new drug development and the modulation of genome stability.

- **What was the impact on technology transfer?** We had filed a provisional patent application in November, 2018 entitled "Electrophiles and electrophile prodrugs as Rad51 inhibitors. New data, described in previous sections, that was supported by the DoD award was included in the updating of this new IP in concert with the international PCT filing on November 27, 2019. We have also formally created, with university COI committee approval, a biotechnology company (Creegh Pharmaceuticals) that has as its primary mission the development of novel drugs for the treatment of drug-resistant cancers.

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

Nothing to report at this time, although from the previous narrative one can see that we intend to further evolve the present research endeavors to directly limit the morbidity and mortality of breast (and other) cancer patients.

5. CHANGES/PROBLEMS:

Nothing to report at this time.

6. PRODUCTS:

Nothing to report at this time.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. **What individuals have worked on the project?**

Name:	Carola A. Neumann
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	3
Contribution to Project:	Dr. Neumann is leading the project and supervising all aspects of the work. Dr. Neumann is directly overseeing Dr. John Skoko, the graduate student Dennis Braden and the research technician Susmita Samanta. Dr. Neumann is organizing regular group meetings with the other participating investigators to discuss and trouble shoot results.
Funding Support:	NIH: R56 CA233817
Name:	John Skoko
Project Role:	Research Instructor

Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	10.8
Contribution to Project:	Dr. Skoko has contributed mostly to the data shown in this report. He is generating CRISPR clones, has characterized different nitro fatty acids for optimal function, viability, drug synergism and will help perform the analysis of the PDX and allograft transplantation models.
Funding Support:	Hillman Foundation funding, Internal
Name:	Dennis Carl Braden
Project Role:	Graduate student
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	6
Contribution to Project:	Mr. Braden has optimized the click reaction for mass spec analysis, and tested cell lines for HDR function and has contributed to cell line viability assays.
Funding Support:	Pharmacology fellowship, internal
Name:	Susmita Samanta
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	1.8
Contribution to Project:	Ms. Samanta is currently testing other TNBC cell lines for viability. No data have been included yet.
Funding Support:	NIH: R56 CA233817

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

8. Other Support

9.

The highlighted award is new since the last reporting

ACTIVE

W81XWH-19-1-0048 (Neumann) 02/01/19-01/31/22 3.00 CM

US Army Department of Defense $1,019,478 TC

Contact: JoAnn Martin, joann.l.martin2.civ@mail.mil, 301-619-2594

Characterizing Nitro-Fatty Acids as Rad51 Inhibitors and Cotreatment in Triple-Negative Breast Cancer

This proposal addresses two overarching challenges: 1) Revolutionizing treatment regimens by replacing them with ones that are more effective, less toxic and impact survival and 2) Conquering the problem of overtreatment.
Role: PD\PI

Grant Number 8117 (Neumann) **01/15/18-01/14/20** **0.24 CM**
Magee Womens Research Institute and Foundation $18,772 TC
Contact: Troy Treanor, treanorta@upmc.edu
Advocating for Clinical Breast Cancer Trials in Allegheny County
Key activities focus on expanding the current UPCI/WCRC/bcRAN program efforts to reduce barriers for patients who would otherwise participate in clinical trials.
Role: PD\PI

SAP 4100079760 (Levine, Oesterreich) **06/01/18-05/31/22** **1.80 CM**
PA Department of Health $706,079 (TC/2 years project)
Contact: Lori Stubbs, Director, HRO PADOH, (717)-231-2825
Estrogen Receptor Dependency in Metastatic Breast Cancer (Tobacco Phase 17 Formula Funds)
The purpose of this project is to develop a mechanistic understanding of ESR1 fusions to provide new concepts for the treatment of endocrine-resistant breast cancer.
Role: Co-I

R56 CA233817 **09/01/19-08/31/21** **3.00 CM**
NCI $687,118 TC
Contact: John R Knowlton, jk339o@nih.gov, 240-276-6210
Inhibition of DNA double strand break repair in TNBC by nitro-fatty acids
The goal of this project to reveal a novel drug strategy for TNBC therapy, where the inhibition of DNA DSB repair by NFAs renders TNBC cells more sensitive to PARP inhibition thus increasing TNBC cell killing.

Role: PD\PI

HCC Development Pilot **01/01/19-12/31/20** **0.00 CM**
HCC $100,745 TC
Development Pilot award
The goal of this project to test if drug synergistic effects exist between NFAs and DNA damaging agents in drug-resistant triple negative breast cancer cells.
Role: PD\PI

PENDING

None

OVERLAP

None

a. **What other organizations were involved as partners?**

Nothing to report at this time.

10. SPECIAL REPORTING REQUIREMENTS
Neumann and Freeman tasks are clearly labelled.

11. APPENDICES
NA