

AWARD NUMBER: W81XWH-20-1-0022

TITLE: Development of New Agents for Treating Endocrine-Resistant Breast Cancer

PRINCIPAL INVESTIGATOR: Wei Xu

CONTRACTING ORGANIZATION: University of Wisconsin System

REPORT DATE: JANUARY 2023

TYPE OF REPORT: Annual Progress Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE JANUARY 2023			2. REPORT TYPE Annual Progress Report			3. DATES COVERED 1JAN2022 - 31DEC2022		
4. TITLE AND SUBTITLE Development of New Agents for Treating Endocrine-Resistant Breast Cancer						5a. CONTRACT NUMBER W81XWH-20-1-0022		
						5b. GRANT NUMBER		
						5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Wei Xu E-Mail: wxu@oncology.wisc.edu						5d. PROJECT NUMBER		
						5e. TASK NUMBER		
						5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Board of Regents of the University of Wisconsin System Research and Sponsored Programs Office 21 N Park St, Ste 6401 Madison, WI 53715						8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012						10. SPONSOR/MONITOR'S ACRONYM(S)		
						11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited								
13. SUPPLEMENTARY NOTES								
14. ABSTRACT Estrogen receptor alpha (ERα) is expressed in ~70% of all human breast cancers and, therefore, is a major therapeutic target for endocrine therapy. The lack of response to anti-estrogens is a hallmark of resistance to endocrine therapies, yet the mechanisms are not completely understood. One emerging mechanism is the development of mutations in <i>ESR1</i> , the gene encoding ERα. These mutant ERα proteins confer significantly higher ERα activity than the wild-type receptor and are resistant to degradation by selective estrogen receptor degraders (SERDs) such as faslodex. Our laboratory discovered a natural plant product, Diptoindonesin G (Dip G), that significantly decreases ERα levels. We determined that Dip G functions via Hsp90α /CHIP, yet the mechanism is different from that of the Hsp90α ATPase inhibitor 17-AAG. Dip G is more effective than faslodex in inhibiting growth of ERα mutant cell lines, with the levels of degradation of ERα, inhibition of ERα target genes and inhibition of cell proliferation all being concordant. Moreover, we have shown that Dip G can effectively inhibit the growth of human tumor organoids. This application will test the hypothesis that Dip G alone or Dip G in combination with SERDs are effective to treat hormone-resistant breast cancers, including those harboring the <i>ESR1</i> mutations.								
15. SUBJECT TERMS NONE LISTED								
16. SECURITY CLASSIFICATION OF:				17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON		
a. REPORT	b. ABSTRACT	c. THIS PAGE		UU	14	USAMRDC		
U	U	U				19b. TELEPHONE NUMBER (include area code)		

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	11
5. Changes/Problems	11
6. Products	12
7. Participants & Other Collaborating Organizations	13
8. Special Reporting Requirements	14
9. Appendices	N/A

1. INTRODUCTION:

Our goal is to develop diptoindonesin G (dip G) into an effective therapeutic drug for treating mutant ER α expressing, endocrine-resistant breast cancers. We will elucidate the mechanism of action of dip G and evaluate the anti-cancer effects of dip G in endocrine-resistant cell lines, organoids, and patient derived xenograft (PDX) models harboring *ESR1* mutations, in comparison with other clinically-investigated ER degrading agents.

2. KEYWORDS:

Endocrine resistance, Estrogen Receptor, Diptoindonesin G, Selective Estrogen Receptor Degradator (SERD), Breast Cancer, Patient-Derived Xenograft

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Goal 1: Mechanistic study of dip G action and the dependency of cytotoxic effects on CHIP, HSP90, and ER protein levels. We have established CHIP KO MCF7 and MCF7-ERY537S cell lines and determined CHIP-dependent and dip G-dependent global protein changes. We also determined binding partners of dip G. A fluorescence polarization assay was established to measure the binding affinity of dip G with recombinant HSP90 and HSP90 fragments, CHIP and ER. We were able to establish that dip G binds with high affinity to the middle domain of HSP90.

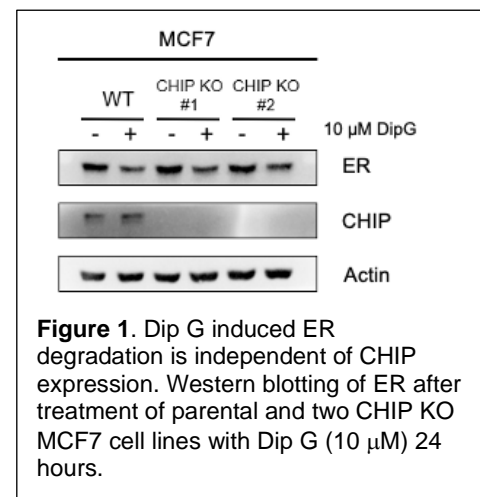
Goal 2: Assess the effects of dip G and SERDs in tumor organoids and MCF7 xenograft mouse models. We have established organoids for WHIM9, 11, 18 and 20, as well as HCI-011, which represent both wildtype ER and mutant ER expressing tumors. We have generated MCF7-luciferase reporter cell lines for xenograft experiments. We treated MCF7-luciferase xenografted mice with dip G and found no significant effects on tumor growth. We treated HCI-011 patient derived organoids with dip G and found that dip G significantly decreased organoid growth, size, and number.

Goal 3: Test the anti-cancer effects of dip G, deoxy-dip G, fulvestrant, and their combination in PDX tumor models. We treated HCI-013 EI (ER Y537S) xenografted mice with deoxy-dip G and found no significant effects on tumor growth. We also tested deoxy-dip G in combination with fulvestrant in WHIM20 (ER Y37S) xenografted mice and found that dip G did not significantly affect tumor growth and did not synergize with fulvestrant. We have characterized the metastatic features of WHIM 9, 11, 18, and 20 by tail-vein injection

What was accomplished under these goals?

(1) Major activities:

Specific Aim 1: Mechanistic study of dip G action and the dependency of cytotoxic effects on CHIP, HSP90 and ER protein level.



Major Task 1: Determine correlation of dip G cytotoxicity with CHIP, HSP70 and ER protein levels. We have successfully generated CHIP KO MCF7 cell lines and measured the dependency of CHIP for dip G-induced ER degradation (Figure 1).

Interestingly, CHIP protein is not required for dip G-induced ER degradation. Though CHIP may still be the primary E3 ligase responsible for dip G-induced ER degradation, a compensatory E3 ligase may promote ER degradation in CHIP's absence.

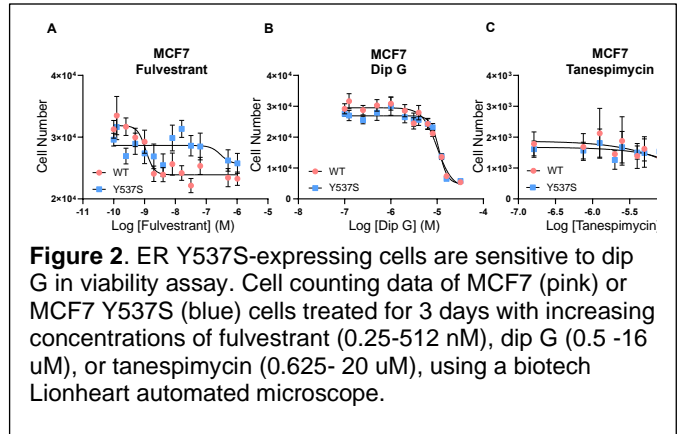


Figure 2. ER Y537S-expressing cells are sensitive to dip G in viability assay. Cell counting data of MCF7 (pink) or MCF7 Y537S (blue) cells treated for 3 days with increasing concentrations of fulvestrant (0.25-512 nM), dip G (0.5 -16 uM), or tanespimycin (0.625- 20 uM), using a biotech Lionheart automated microscope.

We observed a dose-dependent inhibition of cell growth as the concentration of fulvestrant increased in WT MCF7 cells. However, ER Y537S cells were less responsive to fulvestrant treatment. WT ER and ER Y537S cells respond almost identical to dip G treatment, as well as tanespimycin treatment, and this response is dose-dependent (Figure 2). Inhibition of cell growth is correlated with the reduced ER protein levels and ER transcriptional activity (data not shown), indicating that dip G's antiproliferative effects is likely manifested through ER degradation in breast cancer cells.

Major Task 2: Determine the binding of dip G to HSP90/ER/CHIP complex

We have developed fluorescence polarization assay to measure the binding affinity of dip G fluorescent analog to HSP90, CHIP and ER. Interestingly, deoxy-dip G and geldanamycin binding affinity to recombinant HSP90 were measured to be 350 nM and 509 nM, respectively, suggesting that dip G directly binds to HSP90. On the contrary, dip G has weak binding to CHIP and ER recombinant proteins. This result suggests that dip G likely modulates ER protein degradation through binding to HSP90 as a molecular glue to CHIP and ER. Geldanamycin is known to bind to the ATPase domain of HSP90. To determine which domain of HSP90 deoxy-dip G binds to, we expressed and purified GST-tagged HSP90 protein fragments from plasmids corresponding to the N-terminus (AA 9-236), M-domain (AA 272-617), and C-terminus (AA 626-732). We then used thrombin to cleave off the GST tag, and then used these fragments for fluorescence polarization assays. We found that deoxy-dipG's Kd to the middle domain was 130 nM, which is comparable to the value we obtained for deoxy-dip G's binding to full length HSP90 (310 nM), indicating that dip G binds to the middle domain of HSP90, and is a middle domain HSP90 inhibitor. The binding affinities of deoxy-dipG to N- and C-terminal domains of Hsp90 were determined to be 13.8 μ M and 8 μ M, respectively. This further demonstrates that dip G is HSP90 M-domain inhibitor. For this reason, we did not pursue the cytotoxicity experiment with ER overexpression. Rather, we focused on determining to what dip G's binding partners are, as well as Major task 3 to compare the global protein changes by dip G and 17-AAG. To identify dip G's binding partners, Dr. Tang's group synthesized dip G alkyne derivatives to perform CLICK chemistry and pull-down dip G interacting partner proteins using streptavidin beads. Our preliminary experiments identified CHIP and HSP90 in dip G analogue pull-down experiments. We then repeated the pull-down experiments and digested the immunoprecipitants with trypsin, and subjected the samples to proteomics analyses. We found HSP90, as well as other HSP90-related proteins such as HSPA9, HSPB1. We also found UBA1, an E1 ubiquitin activating enzyme.

Major Task 3: Identify CHIP-dependent global protein changes by dip G in MCF7 cells.

We have performed the proteomics analyses comparing dip G with HSP90 inhibitor 17-AAG. Our results showed that dip G regulated proteins significantly overlap and constitute a subset of proteins regulated by 17-AAG. These results further substantiate the mechanism of action of dip G is linked to regulation of HSP90 activity. We then sought to identify CHIP-dependent global protein changes induced by dip G using proteomics. We first measured proteome-wide changes induced by knocking out CHIP. We found 278 proteins were either up- or down-regulated by CHIP KO (Figure 3). We then compared the proteins affected by knocking out CHIP with the proteins affected by dip G treatment. There were 51 proteins affected by both knocking out CHIP and dip G treatment (Figure 3). These proteins are CHIP-dependent dip G changes. However, only about 30% of dip G-induced changes are CHIP-dependent, indicating that many proteins affected by dip G are CHIP-independent, supporting that dip G is an HSP90 inhibitor, and that other E3 ligases function with dip G and HSP90 to regulate stability of client proteins.

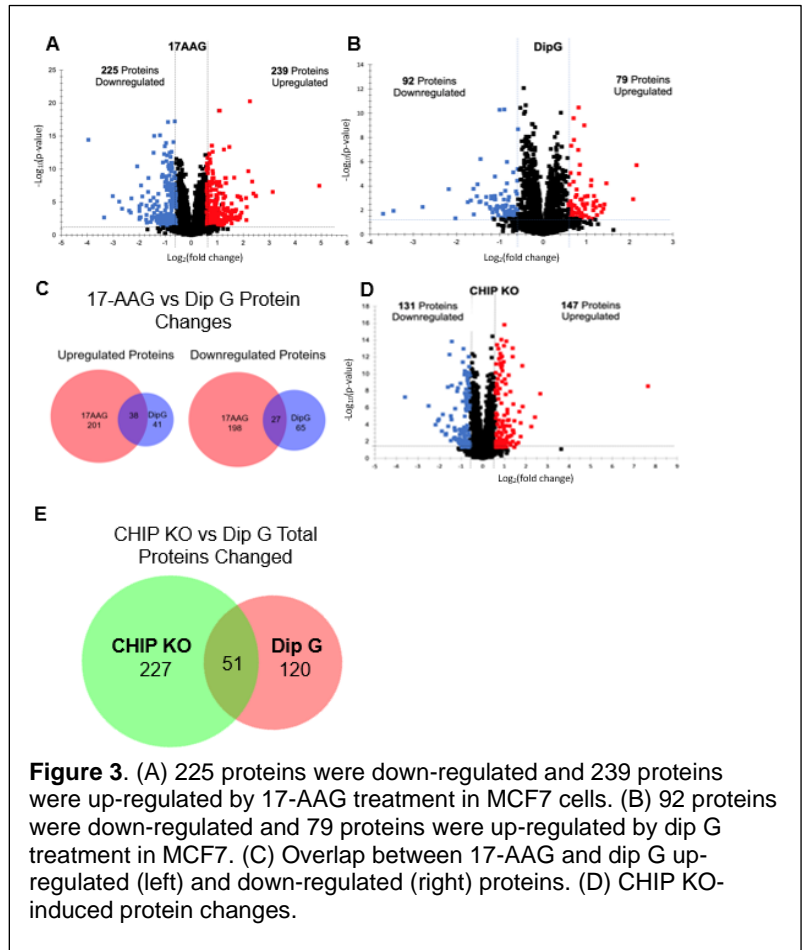
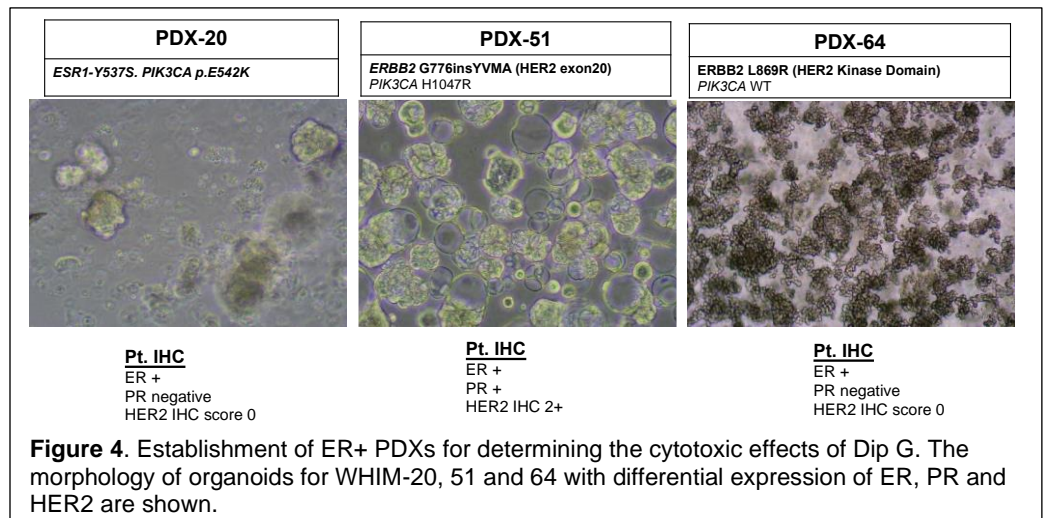


Figure 3. (A) 225 proteins were down-regulated and 239 proteins were up-regulated by 17-AAG treatment in MCF7 cells. (B) 92 proteins were down-regulated and 79 proteins were up-regulated by dip G treatment in MCF7. (C) Overlap between 17-AAG and dip G up-regulated (left) and down-regulated (right) proteins. (D) CHIP KO-induced protein changes. (E) CHIP KO vs Dip G Total Proteins Changed.

Specific Aim 2:
Assess the effects of Dip G and SERDs in tumor organoids and MCF7 xenograft mouse models.

Major Task 4:
Dr. Li has established multiple organoids from ER+ PDXs (Figure 4). WHIM20/PDX20 expresses



ERY537S. These organoids will be used for examining the combinatory effects of Dip G and SERDs.

We have performed viability assays on ER+ HCI-011 PDX-derived organoids from Alana Welm's group. We found using phase contrast microscopy that treatment with 10 μM dip G for one week decreased the number of organoids present (Figure 5). Hsp90 inhibitor tanespimycin and ER degrader fulvestrant were used as positive controls. In addition, many more of the dip G treated organoids were disrupted, and cells were dissociating from each other (Figure 5A). We then performed an MTS assay on these organoids to measure cell viability and found that cell viability in the dip G-treated organoids was significantly decreased ($P < 0.001$) compared to the DMSO treated organoids and were equivalent to tanespimycin or fulvestrant, indicating that HCI-011 PDXOs are sensitive to dip G. (Figure 5B). We next confirmed that Dip G and fulvestrant inhibited expression of ER target gene GREB1 (Figure 5C) and led to degradation of ER (Figure 5D).

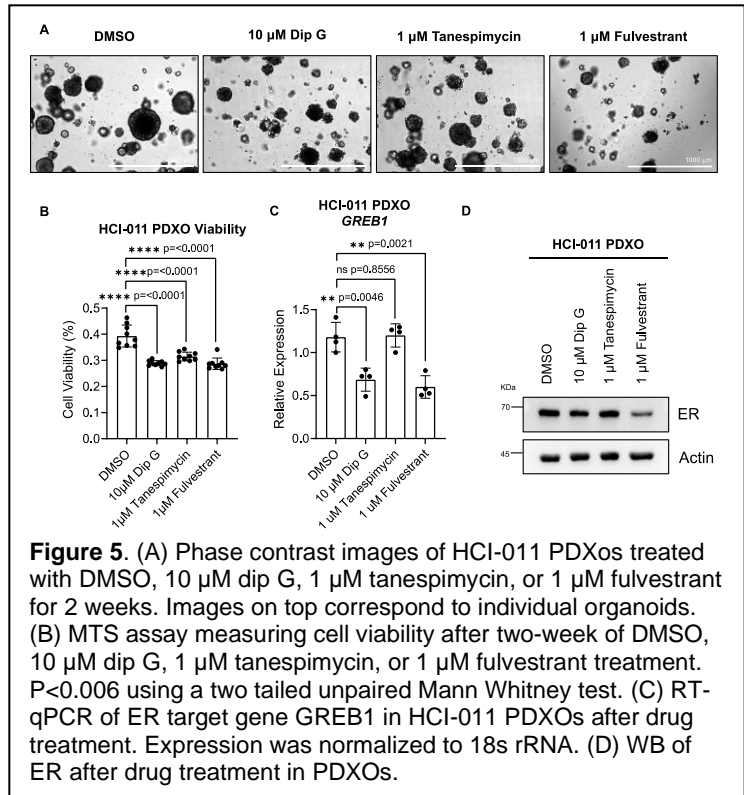


Figure 5. (A) Phase contrast images of HCI-011 PDXOs treated with DMSO, 10 μM dip G, 1 μM tanespimycin, or 1 μM fulvestrant for 2 weeks. Images on top correspond to individual organoids. (B) MTS assay measuring cell viability after two-week of DMSO, 10 μM dip G, 1 μM tanespimycin, or 1 μM fulvestrant treatment. $P < 0.006$ using a two tailed unpaired Mann Whitney test. (C) RT-qPCR of ER target gene GREB1 in HCI-011 PDXOs after drug treatment. Expression was normalized to 18s rRNA. (D) WB of ER after drug treatment in PDXOs.

Major Task 5:

Dr. Li's lab cultured organoids for 10 more PDX models (Figure 6A) and conducted IC_{50} assay on 6 of them in order to identify organoids models that are relatively sensitive to DipG. He will use the "sensitive" models to conduct synergistic study. Organoid cultures were incubated for 2-4 days before the addition of DipG for IC_{50} . DipG was serially diluted in DMSO before the addition to organoid media. Drug concentrations in organoid media were prepared at 2X. Organoid cultures for IC_{50} were exposed to DipG for 5 days. Cell viability was measured with Cell Titer Glo 3D (Promega cat.no G9683) and luminescence was measured using a Tecan infinite M200 plate reader. IC_{50} values (Figure 6B) were calculated using the 4-parameter, non-linear regression function in Graphpad Prism 5.0 for Windows.

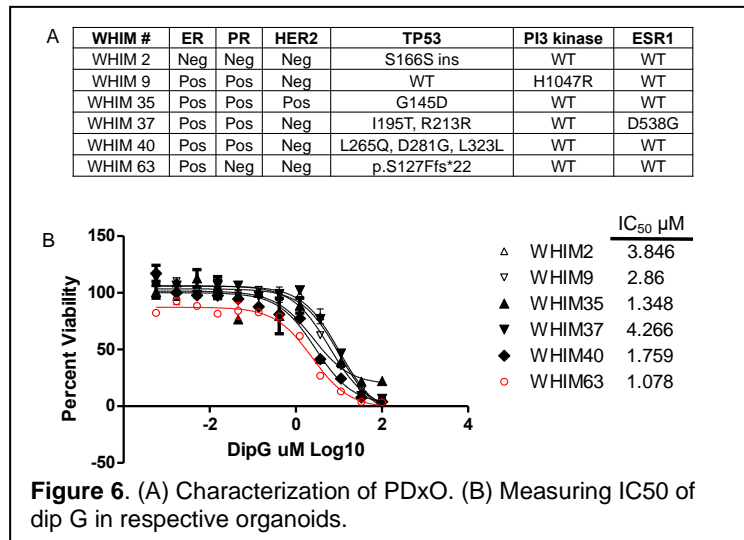
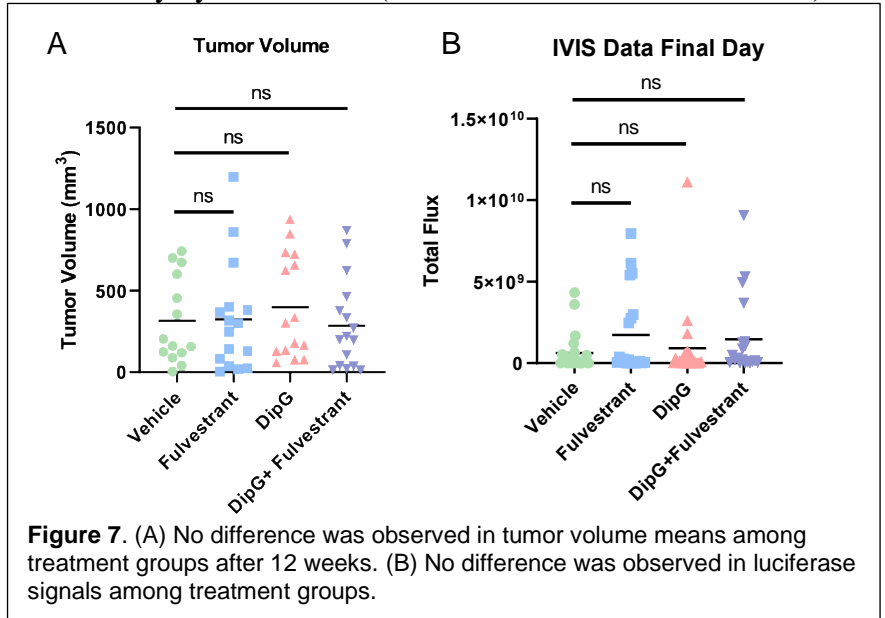


Figure 6. (A) Characterization of PDXO. (B) Measuring IC_{50} of dip G in respective organoids.

Major Task 6:

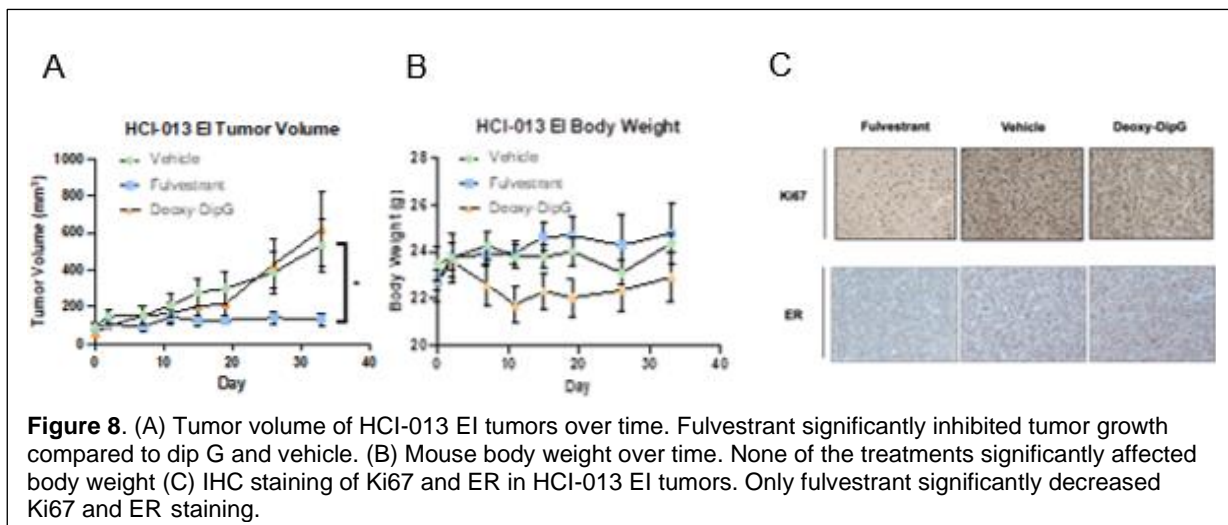
In order to better quantify the effects of drugs on the growth of MCF7 xenograft tumors, we stably expressed GFP-luciferase in MCF7 ERY537S cells. 1×10^6 cells in PBS and Matrigel were injected into the mammary fat pads on either side of each mouse. Tumor size was measured weekly and mice were imaged using IVIS weekly by i.p. injection of luciferin substrate into each mouse (100 ul/mouse) 10 minutes prior to imaging. Both the primary tumors and lungs were imaged. When tumors reached the threshold size for treatment, mice (n=10) were randomized to treatment groups such that the average size for each group was approximately 100 mm^3 . Dip G (40mg/kg in PEG400 & 0.9% saline) was administered daily by s.c. Vehicle (DMSO + PEG400 & 0.9% saline)

treatment group was administered daily by s.c., fulvestrant (150mg/kg in corn oil) was administered weekly by s.c. The treatment lasted for 4 weeks before sacrifice. We did not observe changes in tumor size and luciferase signals in response to treatment regimens among groups (Figure 7). The reason for this remains unclear, because even fulvestrant treatment did not inhibit tumor growth. Because we did not observe any changes in response to dip G treatment in this model, we focused primarily on the PDX model studies, and tested synergy of dip G and SERDs using PDX models.



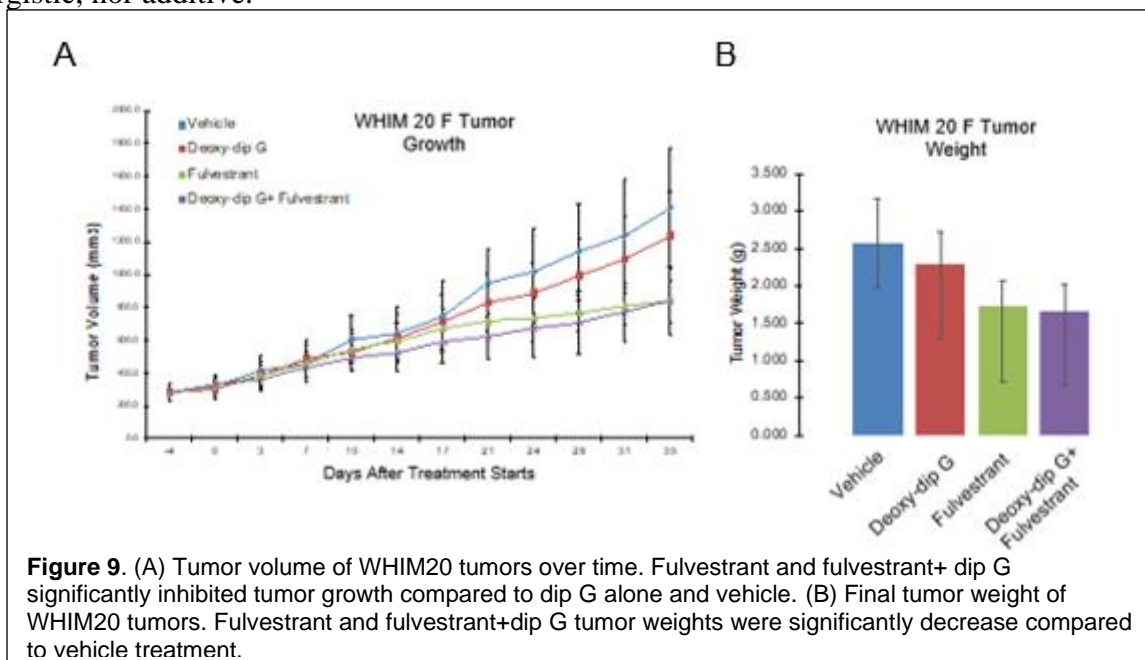
Specific Aim 3: Test Dip G and SERD's effects in PDX tumor models.

Major task 7: Measure in vivo anti-cancer effects of dip G and SERDs in PDX models



We evaluated the anticancer effects of deoxy-dip G, a more potent dip G analog, as well as fulvestrant in PDX model HCI-013 EI (ER Y537S, estrogen independent) in NSG mice. An HCI-013 EI tumor was washed twice with PBS. The tumor was then minced into very small pieces, which were then suspended in PBS and passed through an 18g needle and then a 20g needle to produce a cell suspension for implantation. Matrigel was then added to the suspension in a 1:1 ratio, and the suspension was injected into the fat pads of 6-10 week old NSG mice. Once tumors reached an average size of ~100 mm³, mice were randomized to treatment with 100 mg/kg of deoxy-DipG by oral gavage daily, 125 mg/kg fulvestrant subcutaneously once per week, or vehicle (both DMSO + PEG400 +0.9% saline by oral gavage daily and corn oil + DMSO twice a week). Each group had approximately 6-7 mice/group. Tumor volume was measured by caliper twice a week. Treatment lasted for 33 days, and then mice were sacrificed to harvest tumors. Tumors were formalin fixed and embedded in paraffin. Tissue was sectioned to perform IHC to stain for ER and Ki67. Fulvestrant significantly inhibited the growth of HCI-013 EI tumors compared to vehicle and dip G treatment. Dip G had no significant effect on tumor growth (Figure 8). Though fulvestrant significantly downregulated ER and Ki67 expression compared to vehicle treatment, as measured by immunohistochemistry, dip G had no significant effect (Figure 8).

Next, Dr. Li's group evaluated the anti-tumor effects of deoxy-dip G, fulvestrant, and a combination of deoxy-dip G and fulvestrant to assess synergy of the two compounds, on tumor growth and final tumor weight of ER Y537S-expressing PDX WHIM20. Similarly, Dr. Li's group found that fulvestrant and fulvestrant + deoxy-dip G significantly inhibited the growth of WHIM tumors compared to vehicle and dip G treatment. Dip G had no significant effect on tumor growth compared to vehicle treatment (Figure 9). The growth inhibitory effects seen with the combination treatment can be primarily attributed to fulvestrant, and the combination neither appears to be synergistic, nor additive.



- (2) Specific objectives: Elucidate the mechanism of action of Dip G and investigate the therapeutic effects of Dip G in organoids and PDX models *in vivo*. Significant results and major findings: (a) Towards understanding the mechanism of action of dip G, we performed

fluorescence polarization assays to measure the binding affinity of dip G to recombinant proteins HSP90, CHIP and ER. The results showed that dip G has the strongest binding affinity to HSP90. We found using fragments of HSP90 that deoxy-dip G binds to the middle domain with the highest affinity, indicating that dip G and its analogs are HSP90 middle domain inhibitors. We then performed proteomics analyses comparing dip G and HSP90 inhibitor 17-AAG. The results showed that dip G affected proteins represent a subset of 17-AAG regulated proteome (Figure 3), suggesting that dip G functions through HSP90 but is distinct from 17-AAG. (b) ER degradation still persists in CHIP KO MCF7 cells, suggesting that other ubiquitin ligase E3 may complement CHIP function when CHIP is depleted. Only a small portion of the dip G-affected proteome overlapped with the CHIP KO-affected proteome, indicating that the majority of dip G's affects are not mediated by CHIP. We identified dip G binding partners by mass spectrometry and found HSP90, along with other HSPs. (c) We have attempted to establish MCF7 and patient derived xenograft models for dip G treatment. Unfortunately, we did not observe any growth inhibitory effect by oral gavage or subcutaneous administration of dip G using *in vivo* models. The negative results could be due to insufficient serum and intratumoral levels of dip G, as we see that PDX-derived organoids are sensitive to the concentrations of dip G used in our *in vitro* conditions. (d) we have successfully established several patient derived organoid models, which will be used to identify synergy of dip G with FDA-approved drugs in ER+ organoids using HTS.

(3) Other achievements: None

We have published a paper in JBC as Editors' Pick.

The research activities were impeded by the pandemic. Multiple lab members captured omicron variants and got sick, which compromise the research activities.

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

Donahue K, Xu, W. "Diptoindonesin G, a novel ER alpha degrader for the treatment of endocrine resistant cancer" Oral Presentation, and Poster Presentation. Hormone Dependent Cancers Gordon Research Conference, August 5th, 2019

Kristine Donahue, a graduate student in the Xu lab, has participated in on-campus poster sessions with the Science and Medicine Graduate Research Fellows (SciMed GRS) community.

Kristine Donahue was selected into Heidi Dvinge and Patti Keely Trainee Honor Society in 2020

How were the results disseminated to communities of interest?

Kristine Donahue presented some of the results in two seminars to McArdle laboratory for Cancer Research

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

We have requested no cost extension to perform additional experiments.

Dr. Xu will try to co-crystallize dip G with HSP90. Based on the structure, we will design compounds with improved binding affinity to HSP90. Dr. Li will determine the effect of dip G and

its combination with other FDA-approved drugs in ER+ organoids. We will also use more robust quantitative methods to determine organoid viability to better distinguish between live and dead cells within organoids using calcein AM and ethidium bromide homodimer-1, as well as develop a quantitative way to analyze differences in organoid diameter.

4. IMPACT:

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

The main advantage of dip G comparing to HSP90 ATPase inhibitor such as tanespimycin is that dip G does not induce heat shock response, whereas tanespimycin does. As induction of heat shock response is the major contributor to the development of clinical resistance, dip G appears to be a unique molecule for studying HSP90 biology as well as serving as a new therapeutic avenue for treatment of various cancers.

While endocrine therapy has considerably reduced mortality from breast cancer, resistance to this treatment remains a major clinical challenge. Positive outcomes from these studies will lead to the development of dip G and its analogs as new therapeutic agents for overcoming endocrine-resistance in breast cancers.

What was the impact on other disciplines?

Our proteomics analyses showed that dip G regulates a subset of HSP90 inhibitor 17-AAG-regulated proteins. Dip G does not appear to have strong cytotoxicity in normal cells like N-terminal inhibitors of HSP90. The results suggest that Dip G may substitute HSP90 inhibitors for treatment of multiple human cancer types including those that have developed treatment resistance. Our CHIP KO proteomics data has identified global changes induced by CHIP, which will be useful for identification of novel interactors and clients of CHIP in breast cancer cells.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

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

We completed most of the tasks in SOW.

Administration of Dip G to mice to obtain therapeutically effective dose of the drug remains to be a challenge. We have compared three routes of administration: i.p., subcutaneous, and oral gavage. Almost all administration routes, except for oral gavage, gave low uM concentrations of dip G after 1 hour of administration. We could not detect dip G after oral gavage administration. 1 uM is at the low end of the therapeutically effective dose of dip G in vitro.

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

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Donahue K., Xie, H., Li, M., Gao, A., Ma, M., Wang, Y., Tipton, R., Semanik, N., Primeau, T., Li, S., Li, L., Tang, W., **Xu, W.** (2022) Diptoindonesin G is a middle domain HSP90 modulator for cancer treatment, *Journal of Biological Chemistry*, Nov 14; 102700. This article is selected as ‘Editors’ pick’ and will be featured in JBC. PMID: 36395883

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

Kristine Donahue, Wei Xu. “Therapeutic Strategies to Target Activating Estrogen Receptor alpha Mutations”, *Nuclear Receptors*, SpringerNature Publisher. September 2021.

Other publications, conference papers and presentations.

Donahue K, Xu, W. “Diptoindonesin G, a novel ER alpha degrader for the treatment of endocrine resistant cancer” Oral Presentation, and Poster Presentation. Hormone Dependent Cancers Gordon Research Conference, August 5th, 2019

- **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**

CHIP KO MCF7 cell lines were generated using CRISPR/Cas9

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

WEI XU LAB, UNIVERSITY OF WISCONSIN-MADISON
--

Name: Wei Xu, no change

Name: Yidan Wang, no change

Name: Dr. Ang Gao, no change

Name: Kristine Donahue

Nearest person month worked: 10.0

SHUNQIANG LI LAB, WASHINGTON UNIVERSITY IN ST. LOUIS, MISSOURI

Name: Shunqiang Li, no change

Name: Julie Belmar, no change

Name: Rose Tipton

Nearest person month worked: 1.8

Name: Nicole Semanik

Nearest person month worked: 3.6

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

COLLABORATIVE AWARDS: Shunqiang Li, Partnering PI, will submit a duplicative report.

QUAD CHARTS: N/A

9. APPENDICES: N/A