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# Targeting FASN for breast cancer treatment by repositioning PPIs

## Title:
Targeting FASN for breast cancer treatment by repositioning PPIs

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## ABSTRACT
Human fatty acid synthase (FASN) is the sole cytosolic enzyme responsible for de novo synthesis of palmitate. Recently, it was found that FASN up-regulation contributes to drug and radiation resistance. It has also been found that breast cancers with high level of FASN are 4 times more likely to recur and metastasize than the ones without FASN. We recently found that proton pump inhibitors (PPIs) may inhibit FASN and, thus, possibly can be repurposed as cancer therapeutics. The working hypotheses to be tested in this grant are that FASN over-expression causes drug resistance by up-regulating repair of drug-induced DNA damages and that PPIs may be repositioned as breast cancer drugs by targeting and inhibiting FASN. Three specific aims will be accomplished. In the first year of study, we have shown that FASN up-regulates the expression of SP1 but down-regulates the expression of NF-κB. Both SP1 and NF-κB regulates the expression of PARP1, which, in turn possibly regulates DNA repair activity in response to DNA-damaging drugs such as doxorubicin. We also showed that PPIs dose-dependently inhibit the activity of cellular FASN. In year 2, we will determine how FASN regulates the expression of SP1 and NF-κB and the role of PARP-1 in drug resistance as well as analyze electronic medical records of breast cancer patients for the advantage of PPI use.

## Subject Terms:
FASN, PARP1, SP1, NF-κB, DNA repair, NHEJ, proton pump inhibitors, EMR

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4. INTRODUCTION:
Use of anticancer agents and radiation in appropriate combinations has led to major improvements in the treatment of many malignant tumors. Previously fatal diseases, such as testicular cancer, are now curable and others can undergo meaningful remission. However, many breast cancer patients do not appear to respond well to most chemotherapy strategies and suffer from toxicities of these chemotherapeutic agents. Previously, we found that fatty acid synthase (FASN) up-regulation causes resistance to multiple anticancer drugs including doxorubicine and cisplatin, commonly used breast cancer drugs. These observations are consistent with clinical findings that FASN expression associates with poor prognosis and suggest that FASN upregulation may contribute to the poor outcome in breast cancer treatments. We recently also found that proton pump inhibitors (PPIs) may also inhibit FASN. Thus, the working hypotheses to be tested in this award are that FASN overexpression causes drug resistance by up-regulating repair of drug-induced DNA damages and that PPIs may be repositioned as breast cancer drugs by targeting and inhibiting FASN. Three specific aims will be accomplished to (1) investigate the mechanism of FASN action in drug resistance in breast cancers; (2) determine efficacy of PPIs as single agents and in combinations with doxorubicin in suppressing breast cancer growth in vitro and in vivo; and (3) determine retrospectively the association of PPI usage with outcome of breast cancer patients using bioinformatics approaches and mining electronic medical records.

5. KEYWORDS:
FASN, PARP1, SP1, NF-kB, DNA repair, NHEJ, proton pump inhibitors, EMR, retrospective study

6. ACCOMPLISHMENTS:
For the first year of this grant, we have accomplished most of the tasks as proposed in the original application. The following describes the detailed SOW and what we have accomplished.

3.A. Tasks proposed in SOW for year 1.

Major Task 1: Determine the mechanism of FASN function in breast cancer drug resistance (months 1-16). For this task during the first year, we proposed to establish stable cell lines with FASN over-expression and FASN knockdown and used these cell lines (subtask 1) to determine the role of FASN in regulating NF-kB and SP1 expression (subtask 2) and to determine the role of NF-kB and SP1 in regulating PARP-1 expression (subtask 3).

Major Task 2: Determine the efficacy of PPIs as single agents or in combination with doxorubicin (months 1-14). For this major task during the first year, we plan to determine the effect of stereoisomerization on PPI activity in inhibiting FASN TE (subtask 1) and determine PPI activity in inhibiting cellular FASN and production of free fatty acids (subtask 2).

Major Task 3: Determine association of PPI use with breast cancer outcome by analyzing electronic medical records. For this major task during the first year, we will collect data of EMR (subtask 1) and perform cleansing and conversion of the database to get ready for survival analysis during years 2-3 (subtask 2).

3.B. Summary of Accomplishment in year 1.
The following summarizes our key findings for the subtasks as outlined above.

Major Task 1: Determine the mechanism of FASN function in breast cancer drug resistance. The three subtasks in the major task 1 proposed for year 1 have been accomplished as planned. As shown in Fig. 1A, we have established MCF7 cell line with stable FASN over-expression and MCF7/AdVp3000 cells with stable FASN knockdown as planned. Using these cell line tools, we have shown that FASN plays an important role in up-regulating SP1 but down-regulating NF-kB expression (Fig. 1B and 1C).

Figure 1. Establishment of stable cell lines and FASN regulation of SP1 and p65 expression. A, Western blot analyses of FASN expression in stable MCF7 cells with FASN over-expression (F), MCF7/AdVp3000 cells with stable FASN knockdown (Sh), and their respective control vector-transfected (V) and scrambled control shRNA-transfected (Scr) cells. B and C, Western blot analysis of FASN over-expression (F) and knockdown (Sh) on the expression of NF-kB p65 and SP1 compared with their respective stable clones (V and Scr).
Figure 2. NF-κB and SP1 regulate PARP-1 transcription by binding to the same site. A & B. Effect of SP1 (A) or Flag-p65 (B) over-expression on PARP-1 expression in MCF7 cells as determined by Western blot analyses (left panels) and qRT-PCR (right panels). C. Effect of shRNA-induced p65 knockdown on PARP-1 expression in MCF7 cells as determined using Western blot (left panel) and qRT-PCR (right panel). C Effect of NF-κB activation by TNF-α treatment on PARP-1 expression in MCF7 cells as determined by Western blot.

We have also determined the possible role of NF-κB and SP1 in regulating PARP-1 expression. As shown in Fig. 2A and 2B, SP1 over-expression dramatically increased whereas Flag-p65 (NF-κB) over-expression significantly reduced PARP-1 protein and mRNA levels. Knocking down p65 using shRNA significantly increased PARP-1 protein and mRNA levels (Fig. 2C), whereas activation of p65 with TNF-α had contrary effect (Fig. 2D). Taken together, these results suggest that NF-κB and SP1 oppose each other in regulating PARP-1 transcription with NF-κB functioning as a suppressor and SP1 as an activator.

Major Task 2: Determine the efficacy of PPIs as single agents or in combination with doxorubicin. During the first year of funding, we have accomplished the first two subtasks as planned in the application. Firstly, we tested the isomerization of PPIs (R-lansoprazole vs racemic lansoprazole and S-omeprazole vs racemic omeprazole) using in-vitro 4-MUH fluorogenic assay. Unfortunately, no difference in activity was observed between the R or S isoforms compared with their racemic PPIs (data not shown). Thus, we conclude that isomerization may not have any effect on the PPI activity in inhibiting the thioesterase of human FASN. Secondly, we have determined the effect of PPIs on cellular FASN activity using NADPH oxidation assay of total cell lysate isolated from MCF7 cells. As shown in Fig. 3, all four PPIs dose-dependently inhibited FASN activity with IC50s ranging from 6.7 to 18 μM. Lansoprazole and rabeprazole appear to be the best in inhibiting FASN activity.

Figure 3. PPI inhibition of FASN activity. Particle free cell lysate was prepared from MCF7 cells and was subjected to FASN activity assay using NADPH oxidation assay in the presence of different concentrations of various lansoprazole, omeprazole, pantoprazole, and rabeprazole.

Major Task 3: Determine association of PPI use with breast cancer outcome by analyzing electronic medical records. For this major task, there are two subtasks to be accomplished to collect and cleansing the electronic data during the first year as proposed in the original application. However, accomplishment of these subtasks has been postponed due to the delayed approved of the study on human data by DoD. We have recently been approved on February 17, 2017 for analysis of electronic medical records. We are forging ahead to accomplish these subtasks along with other subtasks proposed for the year 2.

3.C. Future Plans.

In year 2, we will make up the studies to collect data and cleansing them, which were delayed from year 1. We will also accomplish the tasks for year 2 as proposed in our original application. These studies include determining how FASN regulates the expression of SP1 and NF-kB and the role of PARP-1 in drug resistance, determining the effect of PPI on drug sensitivity in animal models, and analyzing electronic medical records of breast cancer patients.

7. IMPACT: Nothing to report during this reporting period.

8. CHANGES/PROBLEMS: Nothing to report during this reporting period.

9. PRODUCTS: Nothing to report during this reporting period.
10. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS. Nothing to report during this reporting period.

11. SPECIAL REPORTING REQUIREMENTS. Nothing to report during this reporting period.

12. APPENDICES. Nothing to report during this reporting period.