Inhibiting Fatty Acid Synthase with Omeprazole to Improve Efficacy of Neoadjuvant Chemotherapy in Patients with Operable TNBC

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ABSTRACT

Purpose: Fatty acid synthase (FASN) is overexpressed in 70% of operable triple-negative breast cancer (TNBC) and is associated with poor prognosis. Proton pump inhibitors selectively inhibit FASN activity and induce apoptosis in TNBC cell lines.

Patients and Methods: Patients with operable TNBC were enrolled in this single-arm phase II study. Patients began omeprazole 80 mg orally twice daily for 4–7 days prior to neoadjuvant anthracycline–taxane-based chemotherapy (AC-T) and continued until surgery. The primary endpoint was pathologic complete response (pCR) in patients with baseline FASN overexpression (FASN+). Secondary endpoints included pCR in all surgery patients, change in FASN expression, enzyme activity, and downstream protein expression after omeprazole monotherapy, safety, and limited omeprazole pharmacokinetics.

Results: Forty-two patients were recruited with a median age of 51 years (28–72). Most patients had ≥cT2 (33, 79%) and ≥N1 (22, 52%) disease. FASN overexpression prior to AC-T was identified in 29 of 34 (85%) evaluable samples. The pCR rate was 72.4% [95% confidence interval (CI), 52.8–87.3] in FASN+ patients and 74.4% (95% CI, 57.9–87.0) in all surgery patients. Peak omeprazole concentration was significantly higher than the IC_{50} for FASN inhibition observed in preclinical testing; FASN expression significantly decreased with omeprazole monotherapy [mean change 0.12 (SD, 0.25); $P = 0.02$]. Omeprazole was well tolerated with no grade ≥ 3 toxicities.

Conclusions: FASN is commonly expressed in early TNBC. Omeprazole can be safely administered in doses that inhibit FASN. The addition of omeprazole to neoadjuvant AC-T yields a promising pCR rate that needs further confirmation in randomized studies.

Introduction

Human fatty acid synthase (FASN) is the sole cytosolic enzyme responsible for *de novo* synthesis of the long-chain fatty acid "palmitate" (1, 2). Under physiologic conditions, FASN is primarily expressed in hormone-sensitive cells, liver, and adipose tissue (1, 3). The typical Western diet contains sufficient free fatty acids so that FASN is not required for normal cell function (3–5). In contrast, cancer cells require *de novo* fatty acid synthesis for survival (1, 6). In addition, FASN induces resistance to multiple DNA-damaging agents including doxorubicin and cisplatin without impacting sensitivity to microtubule agents or antimetabolites (7–9). Inhibition of FASN induces apoptosis selectively in cancer cells both *in vitro* and *in vivo* (10–13) with minimal effect on non-malignant cells (7, 14, 15).

FASN is overexpressed in several epithelial malignancies with over 70% of primary triple-negative breast cancers (TNBC) demonstrating strong FASN expression by IHC (16–21). Breast cancers with high levels of FASN are more likely to recur and metastasize with significantly shorter disease-free and overall survival (16–22). In preclinical studies, we found that the proton pump inhibitors (PPI) effectively downregulate FASN enzyme activity by binding to and inhibiting its thioesterase domain (23), reduce cancer cell survival in TNBC cell lines, and restore sensitivity to anthracycline chemotherapy *in vitro* and *in vivo* (23). PPIs are FDA approved for treatment of a variety of acid-related diseases that plague the digestive system, are widely used, and are well tolerated without major adverse effects (24–27).

There are no approved targeted therapeutic options for patients with operable TNBC (28–30). Anthracycline and taxane–based neoadjuvant chemotherapy (AC-T) is routinely employed for anatomic stage IIA or greater disease (31, 32). Pathologic complete response (pCR; defined as no residual invasive disease in breast and axilla) following neoadjuvant therapy, strongly correlates with improved disease-free and overall survival (32, 33). In contrast, patients with significant residual cancer burden have a higher risk of distant metastases (33, 34). As such, novel approaches can be tested in the neoadjuvant setting using pCR to efficiently identify treatments worthy of more definitive trials that include disease recurrence and survival as endpoints (35, 36).

We report results from a single-arm multi-center phase II trial repurposing high-dose omeprazole as a novel FASN inhibitor in

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Translational Relevance

Human fatty acid synthase (FASN) is the sole cytosolic enzyme responsible for *de novo* synthesis of long-chain saturated fatty acids. FASN is commonly overexpressed in several epithelial malignancies including breast cancers and is associated with poor prognosis. In addition, FASN induces resistance to multiple DNA-damaging agents including doxorubicin and cisplatin. Inhibition of FASN induces apoptosis selectively in cancer cells both *in vitro* and *in vivo* with minimal effect on non-malignant cells. In preclinical studies, we found that the proton pump inhibitors (PPI) effectively downregulate FASN enzyme activity, induce apoptosis in triple-negative breast cancer (TNBC) cell lines, and restore sensitivity to anthracycline chemotherapy *in vitro* and *in vivo*. PPIs are widely used for gastrointestinal disorders and are well tolerated without major adverse effects. Herein, we report pharmacokinetics, biologic activity, and preliminary clinical efficacy of high-dose omeprazole in combination with neoadjuivant chemotherapy in operable TNBC.

combination with standard anthracycline–taxane-based neoadjuvant chemotherapy in operable TNBC (NCT04337580).

Patients and Methods

Patient population

Eligible patients had histologically proven tumor–node–metastasis American Joint Committee on Cancer 7th edition stage II–III invasive, HER2- and hormone receptor–negative breast cancer and were candidates for neoadjuvant anthracycline and taxane–based chemotherapy. Tumors were considered hormone receptor negative if estrogen (ER) and progesterone receptors were present in < 10% of invasive cancer cells by IHC. HER2 overexpression was defined on the basis of ASCO-CAP guidelines (37). In addition, patients were required to have Eastern Cooperative Oncology Group (ECOG) performance status ≤1 with hematologic, renal, hepatic, and cardiac function sufficient to support anthracycline–taxane therapy. Women of childbearing potential underwent pregnancy testing within 14 days of registration and received counseling on the appropriate use of contraception for the duration of study. Prior use of orlistat, a FASN inhibitor with limited systemic absorption, was not allowed. Patients who had used over-the-counter daily PPIs within 6 months or prescription PPIs within 12 months prior to enrollment were excluded. Occasional use of over-the-counter PPIs prior to study entry was allowed. As chronic PPI use may increase bone loss, patients who had had a prior osteoporotic fracture were also excluded.

Treatment plan

Patients were treated with omeprazole, 80 mg orally twice daily, beginning 4–7 days prior to chemotherapy and continuing throughout chemotherapy until the night prior to surgery. Paired biopsy samples were obtained before and after omeprazole monotherapy. After the brief period of omeprazole monotherapy, patients began standard neoadjuvant chemotherapy with doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) for four cycles followed by paclitaxel (80 mg/m^2) weekly for 12 treatments. Doxorubicin and cyclophosphamide (AC) were administered on a classical every 3 week or dose-dense every 2 weeks schedule (with growth factor support) at the treating physician's discretion. Routine incorporation of neoadjuvant carboplatin was not recommended; however, use of carboplatin (AUC 6 on weeks 1, 4, 7, and 10) along with weekly paclitaxel was allowed at the treating investigator's discretion. Prespecified modifications of the chemotherapy dose were permitted to manage the toxic effects of chemotherapy. Dose reductions of omeprazole were not permitted; patients unable to tolerate omeprazole for 14 consecutive days were removed from the study.

Breast imaging was obtained at baseline, following AC and prior to surgery. Patients with overt disease progression during AC moved immediately to paclitaxel therapy. Patients with disease progression during paclitaxel immediately proceeded to definitive surgery. Patients with systemic progression were removed from the study. Decisions regarding the type of surgery (breast conserving surgery, unilateral or bilateral mastectomy), management of the axillary lymph nodes, and incorporation of reconstruction were made according to the patient's preference and treating surgeon's recommendation. Similarly, the use of post-surgery radiotherapy and additional adjuvant systemic therapy were based upon the treating investigator's discretion. Study follow-up continued until definitive surgery or resolution of treatment-related toxicities.

Correlative analyses

FASN expression was assessed on pretreatment tumor samples, but baseline FASN expression was not required for study entry. FASN overexpression (FASN+) was evaluated using Aperio whole slide digital imaging system by IHC (7, 8), reporting the percentage of tumor cells stained with a range from 0% to 100%. Tumors with ≥ 15% aperio positivity were considered FASN+ according to previous reports (38) with modifications considering the sensitivity and specificity of the FASN antibody used in this study. FASN expression and downstream target protein [PARP1 and specificity protein (SP1)] expression were assessed at baseline, days 4–7, and in residual disease at surgery using previously described IHC assays (7, 8, 39, 40). FASN enzyme activity was determined at similar timepoints using previously described coupled enzymatic NADPH oxidation assay (13).

Pharmacokinetics

Given the safety of omeprazole, we chose the highest approved dose of 80 mg orally twice daily to minimize the risk of inadequate exposure in our target population. Plasma samples to quantify R-omeprazole, S-omeprazole, and the corresponding 5′-hydroxy metabolite isomer concentrations were obtained immediately prior to and 2 hours after the first dose. Other samples were serially obtained during planned clinic visits without regard to time from last dose at cycles 2 and 4 of AC and weeks 1 and 7 of paclitaxel. A method to quantify R- and S-omeprazole, and their corresponding 5′-hydroxy metabolites in human plasma, was employed using lansoprazole as the internal standard, liquid–liquid extraction, and HPLC-MS/MS (5500 QTRAP AB Sciex). All stock solutions, internal standard, and samples were protected from light during preparation and analysis.

Study design and statistical analyses

The primary endpoint was pCR, defined as no residual invasive disease in breast or axilla following neoadjuvant therapy, in patients with operable TNBC with baseline FASN overexpression. Secondary endpoints included pCR in all enrolled patients who underwent definitive surgery (surgery population) and biologic activity of high-dose omeprazole as demonstrated by changes in FASN expression, enzyme activity, and downstream target protein expression (NFB, PARP1, and SP1). Safety and limited pharmacokinetics to assess omeprazole exposure were also evaluated.

In the absence of neoadjuvant carboplatin, pCR rates reported with standard A-T backbone from prior studies are highly variable (25%–45%; refs. 33, 41–44). Given substantial differences in response estimates from various studies, we chose a median reported from larger contemporary trials as our null hypothesis, that is, H_0 = pCR rate of approximately 40% (33, 41–44). Assuming that 70% of patients with newly diagnosed TNBC have FASN overexpression, a single-stage phase II trial to detect a pCR rate of 60% with power = 80% and alpha = 0.10 would require 30 FASN-positive patients (42 patients total) and at least 17 of 30 would be required to have a pCR to reject the null hypothesis. pCR rate, for both FASN+ and the surgery population, is reported with 95% exact confidence intervals (CI). Pre- versus post-treatment changes in FASN expression, enzyme activity, and protein expression were compared with two-sided paired *t* tests. Safety was evaluated using Common Terminology Criteria for Adverse Events CTCAE v4.0. Data were summarized with frequency and percent. Continuous measures were summarized by mean, SD, median, minimum, and maximum. All analyses were performed using SAS Version 9.4.

Study oversight

This study was funded by the Breast Cancer Research Foundation. High-dose omeprazole was obtained commercially and provided to study participants without charge. Neoadjuvant chemotherapy was offered as standard of care. The Indiana University Comprehensive Cancer Center (IUSCCC) Clinical Trials Office (A. Bauchle) provided trial management including patient registration, data collection, and quality monitoring. Trial oversight was provided by the IUSCCC Data and Safety Monitoring Committee. All analyses were conducted by the IUSCCC Biostatistics Core (S.K. Althouse, S.M. Perkins) with the exception of the pharmacokinetic analysis, which was conducted by the IUSCCC Clinical Pharmacology Analytics Core (A.R. Masters, R.E. Stratford). The study was conducted in accordance with ethical guidelines consistent with Declaration of Helsinki. Patients provided written informed consent and the protocol was approved by the independent review board at each participating site. All the authors verify that the trial was conducted according to the protocol. All the drafts of the article were prepared and reviewed by the authors for integrity and accuracy of the data.

Results

Patients characteristics

Forty-two women were enrolled from six U.S. centers between January 2016 and May 2019 (**Table 1**). Median age was 51 years (range: 28–72) and 14 (33%) were African American. A majority of patients had tumors > 2 cm (79%) with lymph node involvement (52%). AC was administered in a dose-dense schedule in 86% of patients; 15 patients (36%) received carboplatin along with weekly paclitaxel. Three patients were taken off study after initiating study treatment and prior to surgery (**Fig. 1**) due to disease progression ($n = 1$), illicit drug use and non-compliance ($n = 1$), and after additional testing identified HER 2 amplification ($n = 1$). Thirty-nine patients underwent surgery (surgery population) and 34 patients undergoing surgery had baseline tumor samples available for FASN IHC analyses (FASN population). FASN was overexpressed prior to neoadjuvant chemotherapy in 29 of 34 (85%) patients with evaluable samples with a mean baseline Aperio positivity of 54% (SD, 24%).

Table 1. Patient demographics.

Characteristic	*N* (%)
Race	
Caucasian	24 (57.1)
African American	14 (33.3)
Others	4 (9.5)
Age (years)	
Median	51
Range	28–72
ECOG performance status	
0	36 (85.7)
1	6 (14.3)
Tumor stage	
cT1	8 (19.0)
cT2	27 (64.3)
cT3	6 (14.3)
Unknown	1 (2.4)
Nodal status	
cN0	20 (47.6)
≥N1	22 (53.4)
Neoadjuvant chemotherapy[a]	
Cyclophosphamide/doxorubicin dose dense	36 (85.7)
Carboplatin	15 (35.7)

[a]One patient withdrew before initiating study treatment.

Efficacy

Clinical responses based on breast imaging and/or physical examination were observed in all but 2 patients (5%) during neoadjuvant treatment. The pCR rate in patients with FASN overexpression at study entry was 21/29 = 72.4% (95% CI, 52.8–87.3), exceeding the prespecified boundary of at least 17 with pCR with high-dose omeprazole in combination with AC-T neoadjuvant chemotherapy. The pCR rate in the surgery population was 29/39 = 74.4% (95% CI, 57.9–87.0). The pCR rate in the subset of 15 patients receiving neoadjuvant carboplatin along with weekly paclitaxel was 80% (95% CI, 51.9–95.7).

Safety

High-dose omeprazole was well tolerated with no associated grade (G) 3 or G4 adverse events (**Table 2**). No patient discontinued omeprazole due to toxicity. The most common adverse events attributed to omeprazole included G1 or G2 elevation in liver enzymes (4, 10%), G1 constipation (2, 5%), and hypomagnesemia (1, 2%), all of which resolved after stopping therapy. Neoadjuvant chemotherapy–related toxicity was consistent with previously reported studies with G3 or G4 neutropenia (19%), febrile neutropenia (7%), and peripheral neuropathy (5%) being the most common (**Table 3**). Five patients discontinued chemotherapy early due to toxicity, all within the last 3 weeks of planned therapy. There were no deaths reported in this study.

Correlatives

Paired biosy samples for evaluation of FASN expression before and after omeprazole monotherapy were available in 24 (57%) patients. High-dose omeprazole led to a significant reduction in FASN expression (mean decrease of 12%; SD, 25%; $P = 0.02$; **Fig. 2**). Similarly, FASN enzyme activity significantly decreased with omeprazole monotherapy compared with baseline (mean decrease 0.93; SD, 1.04; $P = 0.01$; **Fig. 3**) in 11 patients that had evaluable paired fresh tissue samples. Omeprazole monotherapy did not significantly modulate expression of proteins downstream from FASN including NFkB [$n =$

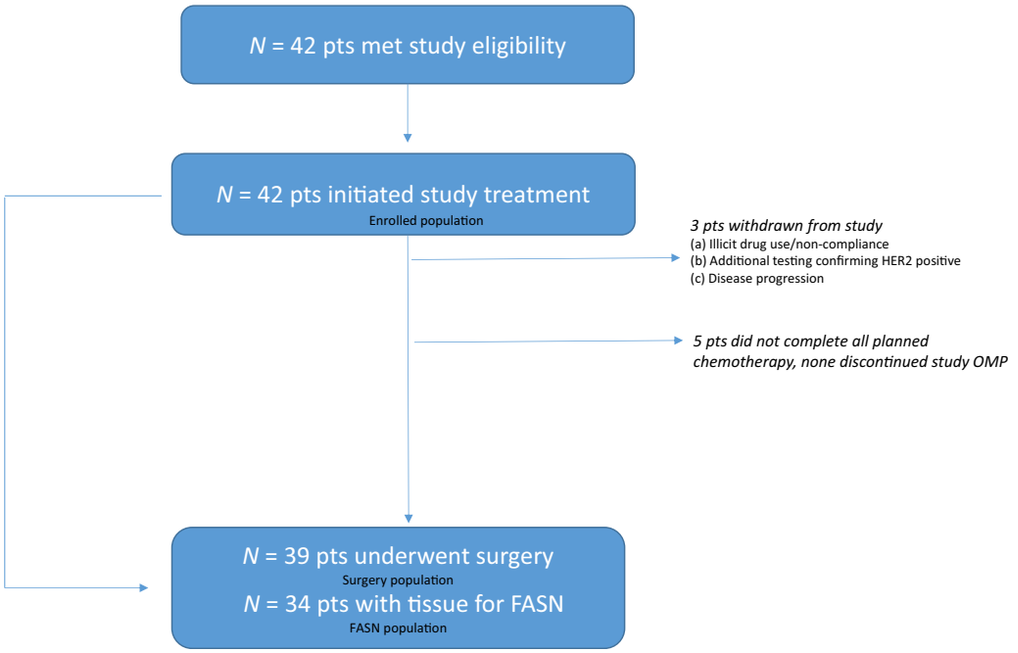

Figure 1.
CONSORT diagram.

25; mean decrease 0.06 (SD, 0.27); $P = 0.28$], PARP-1 [$n = 22$; mean decrease: 0.04 (SD, 0.22); $P = 0.41$], and SP-1 [$n = 24$; mean increase 0.07 (SD, 0.23); $P = 0.15$].

Omeprazole exposure

Average S-omeprazole plasma concentration measured at 2 hours following the first 80 mg racemic omeprazole dose was 522 ± 515.8 ng/mL (mean ± SD; $n = 26$), while average concentration for R-omeprazole was 352 ± 361.2 ng/mL. Although there was substantial variability in these concentrations between patients, S-omeprazole concentrations were consistently higher within individual patients. The average ratio of S-omeprazole to R-omeprazole was 1.9 ± 1.47, which is consistent with a reported ratio of 1.90 following a 40 mg dose of S-omeprazole versus R-omeprazole (45). Concentration at this time for the 5′-hydroxy omeprazole metabolites were 38 ± 37.0 and 189 ± 204.7 for the S- and R-enantiomers, respectively. These differences are consistent with substantially slower intrinsic hepatic clearance of S-omeprazole than the R-enantiomer (46). Upon multiple dosing of racemic omeprazole, no change in the concentrations of its enantiomers and their corresponding 5′-hydroxy metabolite isomers was discernable, suggesting there were no drug interactions between omeprazole and neoadjuvant chemotherapy. The potential for meaningful interactions is low given primarily CYP3A4 and CYPC19 metabolism of S- and R-omeprazole, respectively, and low to absent involvement of these isoforms in metabolism of cyclophosphamide (primarily by CYP2B6), paclitaxel (CYP2C8), and doxorubicin (NADPH dehydrogenases). Peak concentrations of the two omeprazole isoforms following 20 and 40 mg doses were reported to occur at 0.5 hours (45, 46). The half-life of racemic omeprazole in that study was 0.65 hours. The combined average R- plus S-omeprazole concentrations in the current study was 2.1 μmol/L at 2 hours. On the basis of this concentration, the reported half-life of racemic omeprazole and imputing this concentration at the peak of 0.5 hours, results in a predicted peak concentration of 10.4 μmol/L, which is approximately twice the IC_{50} (5.6 μmol/L) for omeprazole inhibition of FASN.

Table 2. Toxicity attributed to omeprazole.

Adverse event	Grade 1 (%)	Grade 2 (%)	Total (%)
Alanine aminotransferase increased	3 (7)	1 (2)	4 (10)
Aspartate aminotransferase increased	2 (5)	0 (0)	2 (5)
Constipation	2 (5)	0 (0)	2 (5)
Headache	2 (5)	0 (0)	2 (5)
Diarrhea	1 (2)	0 (0)	1 (2)
Dry mouth	1 (2)	0 (0)	1 (2)
Fatigue	1 (2)	0 (0)	1 (2)
Hypertriglyceridemia	1 (2)	0 (0)	1 (2)
Hypomagnesemia	1 (2)	0 (0)	1 (2)
Nail ridging	1 (2)	0 (0)	1 (2)
Nausea	1 (2)	0 (0)	1 (2)

Note: No grade 3 or 4 adverse events attributable to omeprazole therapy.

Discussion

FASN overexpression has been implicated in drug resistance and worse prognosis, but there is limited data regarding the prevalence of FASN expression and its association with response to chemotherapy in operable TNBC (22, 47, 48). In a Spanish cohort of 100 patients with early TNBC, FASN was overexpressed in almost all archival samples (92%) and was more frequently associated with node-positive disease and basal-like molecular subtype (21). Our results support these findings with over 80% of tumors showing

Table 3. Toxicity attributed to chemotherapy.

Adverse event	Grade 3 (%)	Grade 4 (%)	Total (%)
Hematologic			
Anemia	5 (12)	0 (0)	5 (12)
Leukopenia	3 (7)	2 (5)	5 (12)
Neutropenia	5 (12)	3 (7)	8 (19)
Febrile neutropenia	3 (7)	0 (0)	3 (7)
Thrombocytopenia	1 (2)	0 (0)	1 (2)
Non-hematologic			
Peripheral neuropathy	2 (5)	0 (0)	2 (5)
Fatigue	1 (2)	0 (0)	1 (2)
Hyperglycemia	3 (7)	0 (0)	3 (7)
Hypokalemia	3 (7)	0 (0)	3 (7)
Constipation	1 (2)	0 (0)	1 (2)
Headache	1 (2)	0 (0)	1 (2)
Mucositis	1 (2)	0 (0)	1 (2)

FASN expression at study entry. FASN thus represents a promising therapeutic target in early TNBC.

It is important to note that FASN overexpression has been observed in other subtypes of breast cancer (49–52) and thus targeting FASN may also benefit these patients (23). We focused on TNBC in this trial for two reasons. First, unlike $HER2^+$ and ER^+ breast cancer, there is no FDA-approved targeted therapy for patients with early-stage TNBC. Chemotherapy remains the mainstay of treatment with routine use of neoadjuvant anthracycline–taxane-based systemic therapy (29–33). Second, neoadjuvant chemotherapy is less frequently used and lack of pCR does not correlate with long-term survival in patients with ER^+ disease (33, 34).

PPIs like omeprazole are effective inhibitors of FASN thioesterase activity (23). PPIs have previously been shown to induce apoptosis and overcome doxorubicin resistance in breast cancer cells, both *in vitro* and *in vivo* (23). In this study, we confirmed that the estimated mean peak exposure after 80 mg orally twice daily omeprazole in patients with early TNBC was significantly higher than the IC_{50} observed during preclinical testing (23). In addition, we demonstrated that omeprazole monotherapy inhibits FASN by significant downregulation of FASN expression and enzyme activity within 4–7 days of initiating therapy. FASN has previously been shown to mediate drug resistance by upregulating PARP-1–mediated non-homologous end joining DNA repair (40). We report a mean decrease in PARP-1 expression with omeprazole monotherapy in this study; however, it did not reach statistical significance. This may be explained by the relatively short 4–7 days duration of FASN inhibition between paired biopsies. Alternatively, activation of other signaling pathways that impact PARP-1 may make FASN inhibition alone insufficient to meaningfully alter protein expression. Finally, we prioritized analyses of FASN expression and enzyme activity, limiting the number of paired samples available for evaluating changes in down stream PARP-1 expression.

The addition of high-dose omeprazole to standard anthracycline–taxane-based chemotherapy led to a pCR rate of 72% in tumors with FASN overexpression at study entry. We observed similar efficacy in intent-to-treat analyses with pCR of 74% in all surgery patients. Preclinical evidence suggests breast cancer cells upregulate FASN over time following exposure to anthracyclines (7). It is possible that high pCR observed in all surgery patients could result from the ability of omeprazole to prevent or overcome acquired resistance after initiating chemotherapy in tumors that were FASN negative at baseline (23). In addition, only a minority (5, 15%) of evaluable samples demonstrated low to none FASN IHC staining at baseline; pCR in this subgroup was not significantly different from pCR in FASN-positive tumors (60% FASN negative vs. 72% FASN positive, $P = 0.62$). Thus, FASN expression may not be required for patient selection. It is important to note that the limited sample size on this single-arm phase II study does not allow for a formal comparison between FASN-positive versus FASN-negative tumors.

It is possible that the antitumor effects of omeprazole seen in our study may not be solely attributable to FASN inhibition. PPIs may also improve chemosensitivity by modulation of intracellular pH via the

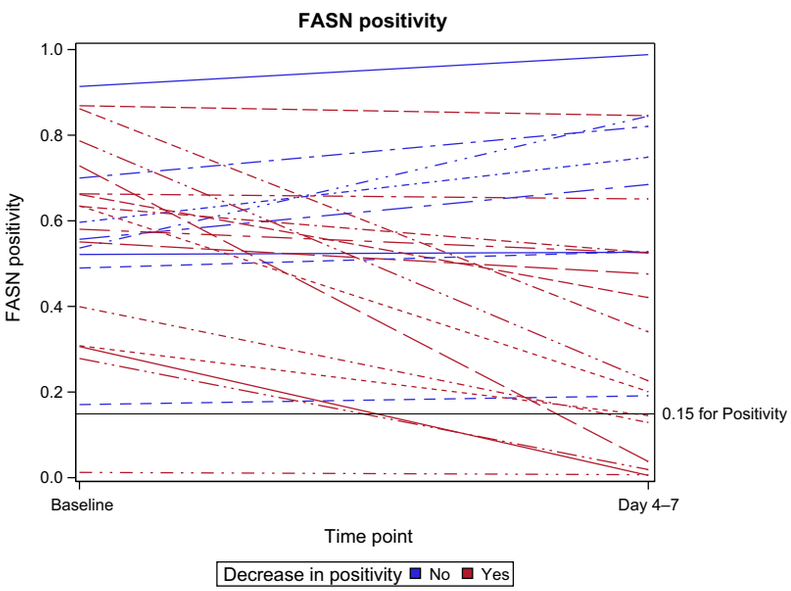

Figure 2.
Change in FASN expression from baseline to days 4–7 with omeprazole monotherapy in $n = 24$ patients with evaluable samples (mean change −0.12; SD, 0.25; $P = 0.02$).

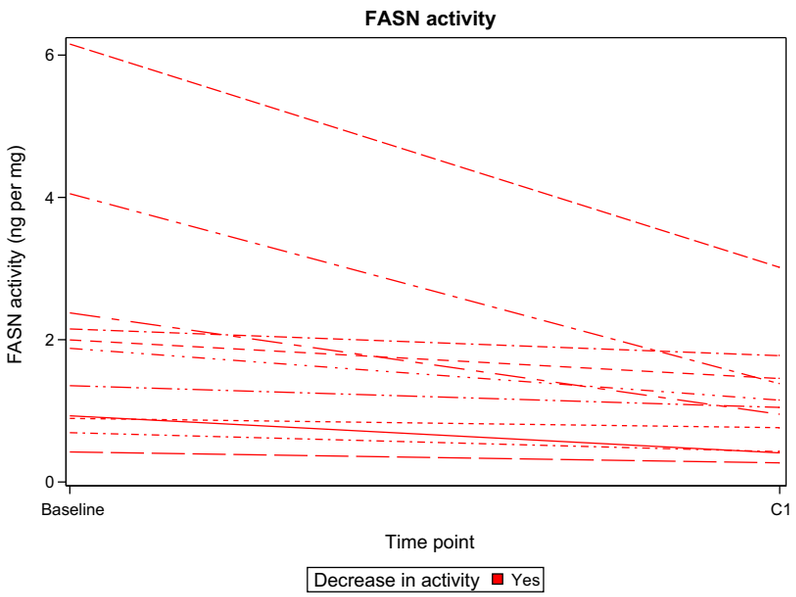

Figure 3.
Change in FASN enzyme activity from baseline to C1 with omeprazole monotherapy in $n = 11$ patients with evaluable fresh tissue samples. All evaluable samples show decrease in FASN activity with a mean decrease of 0.93 (SD, 1.04; $P < 0.01$).

vacuolar H^+ ATPase (V-ATPase) in tumor cells (53, 54). Previous randomized trials in advanced solid tumors have reported improved responses to platinum agents or chemoradiation with the addition of PPIs; however, few have included serial assessment of the biological effects on FASN, V-ATPase activity, or other potential targets (55–57). We did not evaluate alternate pathways that could be associated with a high pCR with the addition of omeprazole in this study. Future trials should continue to focus on biological mechanisms that demonstrate a causal effect between omeprazole-induced FASN inhibition and clinical response.

Many will wonder whether the high pCR rate we report could be attributed to the inclusion of carboplatin in approximately one third of patients (42, 43, 58). *Post hoc* analyses did not reveal a significant difference in pCR rates among patients who received neoadjuvant carboplatin versus not (80% vs. 71%, $P = 0.71$); however, this analysis is limited by small numbers. Future randomized studies should stratify by neoadjuvant platinum use or consider a uniform chemotherapy backbone in this population.

Recent studies report a significant increase in pCR and improvement in event-free survival with the addition of immune checkpoint inhibitors such as atezolizumab or pembrolizumab to neoadjuvant anthracycline–taxane-based chemotherapy (59, 60). However, this improvement comes with increased toxicity and substantial cost. Drug repurposing is a promising strategy that offers the opportunity to reposition "older non-oncology drugs" as effective anti-cancer agents with the advantages of rapid clinical translation, improved cost effectiveness, and, in the case of omeprazole, little added toxicity (61, 62). While synthetic FASN inhibitors are currently being developed in breast cancer therapeutics (NCT03179904), results from our study demonstrate the feasibility of using omeprazole, a widely available and safe generic drug, as a FASN inhibitor. The short duration of high-dose omeprazole did not increase toxicity of neoadjuvant therapy in this study. However, chronic PPI use has been associated with long-term health issues such as osteoporosis, opportunistic infections, and cognitive dysfunction (63). Given the biological implication of FASN inhibition in overcoming chemotherapy resistance, it is unlikely that extended PPI therapy beyond standard duration of cytotoxic therapy would be necessary or provide additional benefit.

In summary, our results confirm the hypothesis that high-dose omeprazole can be safely administered with chemotherapy in concentrations that inhibit FASN in human breast cancers. However, our study is far from definitive and should not impact practice. We chose a single-arm phase II neoadjuvant trial as an initial proof of concept, focusing on pharmacokinetics and biologic activity, while setting an intentionally high bar for clinical activity (pCR). Larger randomized controlled studies evaluating FASN inhibition with omeprazole as a component of neoadjuvant chemotherapy are required to verify the improvement in pCR and survival outcomes we seek, and our patients deserve.

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Authors' Contributions

S.D. Sardesai: Conceptualization, resources, investigation, writing–original draft, writing–review and editing. **A. Thomas:** Investigation, writing–review and editing. **C. Gallagher:** Writing–review and editing. **F. Lynce:** Investigation, writing–review and editing. **Y.L. Ottaviano:** Investigation, writing–review and editing. **T.J. Ballinger:** Investigation, writing–review and editing. **B.P. Schneider:** Investigation, writing–review and editing. **A.M. Storniolo:** Investigation, writing–review and editing. **A. Bauchle:** Project administration. **S.K. Althouse:** Software, formal analysis, methodology, writing–review and editing.

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References

1. Liu H, Liu JY, Wu X, Zhang J-T. Biochemistry, molecular biology, and pharmacology of fatty acid synthase, an emerging therapeutic target and diagnosis/prognosis marker. Int J Biochem Mol Biol 2010;1:69–89.
2. Kuhajda FP. Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. Nutrition 2000;16:202–8.
3. Kusakabe T, Maeda M, Hoshi N, Sugino T, Watanabe K, Fukuda T, et al. Fatty acid synthase is expressed mainly in adult hormone-sensitive cells or cells with high lipid metabolism and in proliferating fetal cells. J Histochem Cytochem 2000;48:613–22.
4. Sul HS, Wang D. Nutritional and hormonal regulation of enzymes in fat synthesis: studies of fatty acid synthase and mitochondrial glycerol-3-phosphate acyltransferase gene transcription. Annu Rev Nutr 1998;18:331–51.
5. Anderson SM, Rudolph MC, McManaman JL, Neville MC. Key stages in mammary gland development. Secretory activation in the mammary gland: it's not just about milk protein synthesis! Breast Cancer Res 2007;9:204.
6. Kuhajda FP, Jenner K, Wood FD, Hennigar RA, Jacobs LB, Dick JD, et al. Fatty acid synthesis: a potential selective target for antineoplastic therapy. Proc Natl Acad Sci U S A 1994;91:6379–83.
7. Liu H, Liu Y, Zhang JT. A new mechanism of drug resistance in breast cancer cells: fatty acid synthase overexpression-mediated palmitate overproduction. Mol Cancer Ther 2008;7:263–70.
8. Liu H, Wu X, Dong Z, Luo Z, Zhao Z, Xu Y, et al. Fatty acid synthase causes drug resistance by inhibiting TNF-alpha and ceramide production. J Lipid Res 2013;54:776–85.
9. Yang Y, Liu H, Li Z, Zhao Z, Yip-Schneider M, Fan Q, et al. Role of fatty acid synthase in gemcitabine and radiation resistance of pancreatic cancers. Int J Biochem Mol Biol 2011;2:89–98.
10. De Schrijver E, Brusselmans K, Heyns W, Verhoeven G, Swinnen JV. RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells. Cancer Res 2003;63:3799–804.
11. Zhou W, Simpson PJ, McFadden JM, Townsend CA, Medghalchi SM, Vadlamudi A, et al. Fatty acid synthase inhibition triggers apoptosis during S phase in human cancer cells. Cancer Res 2003;63:7330–7.
12. Menendez JA, Colomer R, Lupu R. Inhibition of tumor-associated fatty acid synthase activity enhances vinorelbine (Navelbine)-induced cytotoxicity and apoptotic cell death in human breast cancer cells. Oncol Rep 2004;12:411–22.
13. Menendez JA, Mehmi I, Atlas E, Colomer R, Lupu R. Novel signaling molecules implicated in tumor-associated fatty acid synthase-dependent breast cancer cell proliferation and survival: Role of exogenous dietary fatty acids, p53-p21WAF1/CIP1, ERK1/2 MAPK, p27KIP1, BRCA1, and NF-kappaB. Int J Oncol 2004;24:591–608.
14. Browne CD, Hindmarsh EJ, Smith JW. Inhibition of endothelial cell proliferation and angiogenesis by orlistat, a fatty acid synthase inhibitor. FASEB J 2006;20:2027–35.
15. Chajes V, Cambot M, Moreau K, Lenoir GM, Joulin V. Acetyl-CoA carboxylase alpha is essential to breast cancer cell survival. Cancer Res 2006;66:5287–94.
16. Alo PL, Visca P, Marci A, Mangoni A, Botti C, Di Tondo U. Expression of fatty acid synthase (FAS) as a predictor of recurrence in stage I breast carcinoma patients. Cancer 1996;77:474–82.
17. Esslimani-Sahla M, Thezenas S, Simony-Lafontaine J, Kramar A, Lavaill R, Chalbos D, et al. Increased expression of fatty acid synthase and progesterone receptor in early steps of human mammary carcinogenesis. Int J Cancer 2007;120:224–9.
18. Epstein JI, Carmichael M, Partin AW. OA-519 (fatty acid synthase) as an independent predictor of pathologic state in adenocarcinoma of the prostate. Urology 1995;45:81–6.
19. Gansler TS, Hardman W 3rd, Hunt DA, Schaffel S, Hennigar RA. Increased expression of fatty acid synthase (OA-519) in ovarian neoplasms predicts shorter survival. Hum Pathol 1997;28:686–92.
20. Alo PL, Amini M, Piro F, Pizzuti L, Sebastiani V, Botti C, et al. Immunohistochemical expression and prognostic significance of fatty acid synthase in pancreatic carcinoma. Anticancer Res 2007;27:2523–7.
21. Giro-Perafita A, Sarrats A, Perez-Bueno F, Oliveras G, Buxó M, Brunet J, et al. Fatty acid synthase expression and its association with clinicohistopathological features in triple-negative breast cancer. Oncotarget 2017;8:74391–405.
22. Alo PL, Visca P, Trombetta G, Mangoni A, Lenti L, Monaco S, et al. Fatty acid synthase (FAS) predictive strength in poorly differentiated early breast carcinomas. Tumori 1999;85:35–40.
23. Wang CJ, Li D, Danielson JA, Zhang EH, Dong Z, Miller KD, et al. Proton pump inhibitors suppress DNA damage repair and sensitize treatment resistance in breast cancer by targeting fatty acid synthase. Cancer Lett 2021;509:1–12.
24. Sachs G. Proton pump inhibitors and acid-related diseases. Pharmacotherapy 1997;17:22–37.
25. Stedman CA, Barclay ML. Review article: comparison of the pharmacokinetics, acid suppression and efficacy of proton pump inhibitors. Aliment Pharmacol Ther 2000;14:963–78.
26. Shin JM, Kim N. Pharmacokinetics and pharmacodynamics of the proton pump inhibitors. J Neurogastroenterol Motil 2013;19:25–35.
27. Ali Khan M, Howden CW. The role of proton pump inhibitors in the management of upper gastrointestinal disorders. Gastroenterol Hepatol 2018;14:169–75.
28. Mehanna J, Haddad FG, Eid R, Lambertini M, Kourie HR. Triple-negative breast cancer: current perspective on the evolving therapeutic landscape. Int J Womens Health 2019;11:431–7.
29. Li CH, Karantza V, Aktan G, Lala M. Current treatment landscape for patients with locally recurrent inoperable or metastatic triple-negative breast cancer: a systematic literature review. Breast Cancer Res 2019;21:143.
30. Wahba HA, El-Hadaad HA. Current approaches in treatment of triple-negative breast cancer. Cancer Biol Med 2015;12:106–16.
31. Hayes DF, Schott AF. Neoadjuvant chemotherapy: what are the benefits for the patient and for the investigator? J Natl Cancer Inst Monogr 2015;2015:36–9.
32. von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012;30:1796–804.
33. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 2014;384:164–72.
34. Symmans WF, Wei C, Gould R, Yu X, Zhang Y, Liu M, et al. Long-term prognostic risk after neoadjuvant chemotherapy associated with residual cancer burden and breast cancer subtype. J Clin Oncol 2017;35:1049–60.
35. Prowell TM, Pazdur R. Pathological complete response and accelerated drug approval in early breast cancer. N Engl J Med 2012;366:2438–41.
36. Barker AD, Sigman CC, Kelloff GJ, Hylton NM, Berry DA, Esserman LJ. I-SPY 2: an adaptive breast cancer trial design in the setting of neoadjuvant chemotherapy. Clin Pharmacol Ther 2009;86:97–100.

37. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol 2018;36: 2105–22.
38. Visca P, Alò PL, Del Nonno F, Botti C, Trombetta G, Marandino F, et al. Immunohistochemical expression of fatty acid synthase, apoptotic-regulating genes, proliferating factors, and ras protein product in colorectal adenomas, carcinomas, and adjacent nonneoplastic mucosa. Clin Cancer Res 1999;5: 4111–8.
39. Chen Q, Yang Y, Li L, Zhang J-T. The amino terminus of the human multidrug resistance transporter ABCC1 has a U-shaped folding with a gating function. J Biol Chem 2006;281:31152–63.
40. Wu X, Dong Z, Wang CJ, Barlow LJ, Fako V, Serrano MA, et al. FASN regulates cellular response to genotoxic treatments by increasing PARP-1 expression and DNA repair activity via NF-kappaB and SP1. Proc Natl Acad Sci U S A 2016;113: E6965–73.
41. Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahlenberg MS, Robidoux A, et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. J Clin Oncol 2008;26:778–85.
42. Sikov WM, Berry DA, Perou CM, Singh B, Cirrincione CT, Tolaney SM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week followed by dose-dense doxorubicin and on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). J Clin Oncol 2015;33:13–21.
43. Loibl S, O'Shaughnessy J, Untch M, Sikov WM, Rugo HS, McKee MD, et al. Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BrighTNess): a randomised, phase 3 trial. Lancet Oncol 2018;19:497–509.
44. Mieog JS, van der Hage JA, van de Velde CJ. Preoperative chemotherapy for women with operable breast cancer. Cochrane Database Syst Rev 2007;2007: CD005002.
45. Hassan-Alin M, Andersson T, Niazi M, Röhss K. A pharmacokinetic study comparing single and repeated oral doses of 20 mg and 40 mg omeprazole and its two optical isomers, S-omeprazole (esomeprazole) and R-omeprazole, in healthy subjects. Eur J Clin Pharmacol 2005;60:779–84.
46. Abelö A, Andersson TB, Antonsson M, Naudot AK, Skånberg I, Weidolf L. Stereoselective metabolism of omeprazole by human cytochrome P450 enzymes. Drug Metab Dispos 2000;28:966–72.
47. Wu X, Qin L, Fako V, Zhang J-T. Molecular mechanisms of fatty acid synthase (FASN)-mediated resistance to anti-cancer treatments. Adv Biol Regul 2014;54: 214–21.
48. Giro-Perafita A, Palomeras S, Lum DH, Blancafort A, Viñas G, Oliveras G, et al. Preclinical evaluation of fatty acid synthase and EGFR inhibition in triple-negative breast cancer. Clin Cancer Res 2016;22:4687–97.
49. Jin Q, Yuan LX, Boulbes D, Baek JM, Wang YN, Gomez-Cabello D, et al. Fatty acid synthase phosphorylation: a novel therapeutic target in HER2-overexpressing breast cancer cells. Breast Cancer Res 2010; 12:R96.
50. Menendez J, Lupu R. Fatty acid synthase regulates estrogen receptor-α signaling in breast cancer cells. Oncogenesis 2017;6:e299.
51. Xu S, Chen T, Dong L, Li T, Xue H, Gao B, et al. Fatty acid synthase promotes breast cancer metastasis by mediating changes in fatty acid metabolism. Oncol Lett 2021;21:27.
52. Fako VE, Wu X, Pflug B, Liu J-Y, Zhang J-T. Repositioning proton pump inhibitors as anticancer drugs by targeting the thioesterase domain of human fatty acid synthase. J Med Chem 2015;58:778–84.
53. Stransky L, Cotter K, Forgac M. The function of V-ATPases in cancer. Physiol Rev 2016;96:1071–91.
54. Feng S, Zhu G, McConnell M, Deng L, Zhao Q, Wu M, et al. Silencing of atp6v1c1 prevents breast cancer growth and bone metastasis. Int J Biol Sci 2013;9:853–62.
55. Luciani F, Spada M, De Milito A, Molinari A, Rivoltini L, Montinaro A, et al. Effect of proton pump inhibitor pretreatment on resistance of solid tumors to cytotoxic drugs. J Natl Cancer Inst 2004;96:1702–13.
56. Wang BY, Zhang J, Wang JL, Sun S, Wang ZH, Wang LP, et al. Intermittent high dose proton pump inhibitor enhances the antitumor effects of chemotherapy in metastatic breast cancer. J Exp Clin Cancer Res 2015;34:85.
57. Ikemura K, Hiramatsu S, Okuda M. Drug repositioning of proton pump inhibitors for enhanced efficacy and safety of cancer chemotherapy. Front Pharmacol 2017;8:911.
58. von Minckwitz G, Schneeweiss A, Loibl S, Salat C, Denkert C, Rezai M, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. Lancet Oncol 2014;15:747–56.
59. Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, et al. Pembrolizumab for early triple-negative breast cancer. N Engl J Med 2020; 382:810–21.
60. Mittendorf EA, Zhang H, Barrios CH, Saji S, Jung KH, Hegg R, et al. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. Lancet 2020;396:1090–100.
61. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, et al. Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 2019;18:41–58.
62. Pollak M. Overcoming drug development bottlenecks with repurposing: repurposing biguanides to target energy metabolism for cancer treatment. Nat Med 2014;20:591–3.
63. Jaynes M, Kumar AB. The risks of long-term use of proton pump inhibitors: a critical review. Ther Adv Drug Saf 2019;10:2042098618809927.