ADAM-17: a novel therapeutic target for triple negative breast cancer

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Background: Validated targeted therapy is currently unavailable for patients with invasive breast cancer negative for oestrogen receptors, progesterone receptors and HER2 [i.e., those with triple-negative (TN) disease]. ADAM-17 is a protease involved in the activations of several ligands that bind to and promotes intracellular signalling from the EGFR/HER family of receptors.

Patients and methods: Expression of ADAM-17 was measured in 86 triple-negative and 96 non-triple-negative breast cancers. The ADAM-17 specific inhibitor, PF-5480090 (TMI-002, Pfizer) was tested in a panel of breast cancer cell lines for effects on functional outputs.

Results: In this study we show using both Western blotting and immunohistochemistry that ADAM-17 is expressed at significantly higher levels in TN than non-TN breast cancers. Using a panel of breast cancer cell lines in culture, PF-5480090 was found to decrease release of the EGFR ligand, TGF-alpha, decrease levels of phosphorylated EGFR and block cell proliferation in a cell-type-dependent manner. Potentially important was the finding of a significant and moderately strong correlation between ADAM-17 activity and extent of proliferation inhibition by PF-5480090 (r = 0.809; p = 0.003; n = 11). Pretreatment of cell lines with PF-5480090 enhanced response to several different cytotoxic and anti-EGFR/HER agents.

Conclusion: It is concluded that inhibition of ADAM-17, especially in combination with chemotherapy or anti-EGFR/HER inhibitors, may be a new approach for treating breast cancer, including patients with TN disease.

Key words: ADAM17, therapeutics, triple-negative breast cancer

Introduction

Using gene expression profiling, several different subforms of invasive breast cancer have been identified including luminal A, luminal B, HER2 type and basal type [1, 2]. Approximately 80% of the basal type breast cancers are negative for oestrogen receptor, progesterone receptors and HER2, using routine clinical assays. Patients lacking these three biomarkers have been dubbed triple negative (TN). Although only approximately 15% of invasive breast cancers are TN, triple negativity is responsible for a greater proportion of deaths from this malignancy [3]. This high death rate appears to be due to the intrinsic aggressiveness and lack of targeted therapy. Currently, there is therefore intense interest in developing new targeted therapies for this form of breast cancer [3].

Although HER2 is not overexpressed in TN breast cancer, another member of the HER family, i.e. epidermal growth factor receptor (EGFR) has been implicated in this molecular subform of breast cancer. Thus, in vitro studies show more active EGFR-RAS signalling [4, 5] and greater sensitivity to anti-EGFR agents in TN/basal cells lines than in luminal cell lines [4, 6]. Furthermore, high levels of EGFR in TN breast cancers have been associated with the poor outcome [6]. All of these findings, when taken together, suggests that EGFR signalling may be involved in either the formation or the progression of at least a subset of TN breast cancers.

The activation of EGFR is normally mediated by the release of ligands such as transforming growth factor-alpha (TGF-α), amphiregulin, EGF, betacellulin, epiregulin and heparin binding (HB)-EGF [7–9]. The release of these ligands is catalyzed by two ADAM proteases, i.e. ADAM-10 and ADAM-17. Thus, ADAM-17 is the principal sheddase for TGF-α, amphiregulin, epiregulin and HB-EGF, while ADAM-10 has been shown to be responsible for the release of EGF and betacellulin [7–9].
Since ADAM-17 is necessary for the release of several of the EGFR activating ligands, we hypothesized that the blockage of its protease activity may be a new approach to treating TN breast cancer. The aim of this investigation was therefore to establish that ADAM-17 was present in TN breast cancer cells in culture. First, however, we aimed to establish that ADAM-17 was present in TN breast cancers.

**materials and methods**

**ADAM-17 mRNA expression in the different molecular subforms of breast cancer: use of a pooled database**

ADAM-17 mRNA expression in the different molecular subforms of breast cancer was determined using a pooled database. Gene expression datasets were downloaded from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) in the form of raw data files. Only datasets with at least 50 patients, raw data available and incorporating the Affymetrix U133A or U133 plus 2.0 platforms were included. Twelve datasets containing 2060 patients were found to be suitable for analysis (supplementary Table S2, available at *Annals of Oncology* online). Gene expression values were called using the robust multichip average method [12] and data were quantile normalized using the Bioconductor package, affy (www.bioconductor.org). Hybridization probes were mapped to Entrez gene IDs [13]. Probes that hit multiple genes were filtered out. When multiple probes for the same gene were used, the probes were averaged for that gene. The R package Genefu was used to classify the 2335 breast cancer samples into their molecular subtypes, i.e. luminal A, luminal B, HER2-positive and basal, using the ssp2003 classifier [14]. All calculations were carried out in the R statistical environment (http://cran.r-project.org/).

Adjusting for batch effect in the gene expression analysis was carried out using the ComBat software [15]. Affymetrix U133A or Affymetrix U133 Plus 2.0 platforms were analyzed as they are the most commonly used platforms and share ∼22 000 probes. This allowed us to combine the largest number of samples (2335 samples from 12 datasets). As adjusting for batch effect changes the expression levels in the individual samples, we compared the relative expression levels of ADAM-17 as opposed to absolute expression differences, among the different breast cancer subtypes. The relative expression levels of ADAM-17 among the breast cancer subtypes were compared using an analysis of variance. These calculations were also carried out in the R statistical environment (http://cran.r-project.org/).

**protein isolation and immunoblotting**

Human breast cancer samples were obtained following institutional board ethical approval from St. Vincent’s University Hospital. Tissues were snap frozen in liquid nitrogen and stored at −80°C, following surgical resection and pathological evaluation. Tissue was extracted and subjected to western blotting using rabbit polyclonal anti-TACE antibody (ProSci Inc, Poway, CA) as previously described [16]. Supplementary Table S1, available at *Annals of Oncology* online summarizes the characteristics of the primary breast carcinomas analyzed by western blotting.

**immunohistochemistry**

Staining for ADAM-17 was carried out with the above mentioned antibody (5 μg/ml), using the NovoCastra Novolink polymer detection system, according to the manufacturer’s instructions (NovoCastra, Newcastle, UK). The tissue microarrays used and corresponding tumour characteristics were as previously described (and are summarized in supplementary Table S1, available at *Annals of Oncology* online) [17]. Rabbit IgG control antibodies were used as negative controls. Slides were incubated with secondary antibody/HRP (DAKO, Glostrup, Denmark). 3,3’-Diaminobenzidine (DAB; 1:50 dilution) was used to visualize the staining. Mayer’s haematoxylin (BDH Laboratories, Poole, UK) was incubated for 30 s as a counterstain before mounting in diystrene, plasticizer and xylene mounting media (BDH). Images were captured using the Olympus DP50 light microscope and AnalySIS software (Soft Imaging System Corporation, Lakewood, CO, USA). Slides were scored for cytoplasmic staining, as following: 0 = no staining, 1 = weak intensity, 2 = moderate intensity and 3 = high intensity. Staining was evaluated and scored by two independent investigators. Discrepancies were resolved to achieve the concordance. Level 3 staining intensity was arbitrarily selected as containing high levels of ADAM-17.

**cell viability assays**

Cell proliferation was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Cell Proliferation Kit I (Roche Applied Science) or direct cell counting. To test the effect of PF-5480090 on proliferation alone or in combination with anti-HER/chemotherapy agents, cells were plated at a density of 1 × 10⁴/well in 96-well flat-bottomed plates (Corning® Costar®, Sigma-Aldrich, St. Louis, USA). Following overnight incubation, triplicate wells were treated with varying concentrations of compounds alone or in combination, for 5 days. Cell viability was determined using the MTT cell proliferation kit I (Roche), and absorbance was measured at a wavelength of 550 nm on a microplate reader (Multiscan Ascent, Labsystems). For direct counting, cells were plated at a density of 5 × 10⁴/well in six-well plates. Following overnight incubation, wells in each plate were treated with compound or vehicle for 5 days. Cells were trypsinized and centrifuged at 1000g for 5 min. The supernatant was removed and pellet resuspended into culture medium. The samples of cell suspension were diluted 1:2 in trypan blue, and viable cells were counted using the Countess® Automated Cell Counter (Invitrogen Life Technologies, Carlsbad, CA, USA).

**clonogenic assays**

Cells were seeded in six-well plates at a density of 1 × 10⁴ cells per well in triplicate and treated with compound for 7–14 days (cell line dependent). Cells were fixed in 1% glutaraldehyde (Sigma) and stained with 0.1% crystal violet (Pro-Lab Diagnostics, Merseyside, UK). The mean colony count and standard error of the mean were calculated.

**other assays**

TGF-β protein levels were determined by ELISA (R&D Systems, Abingdon, UK), ADAM-17 catalytic activity by the InnoZyme TACE Activity kit (Merck, Darmstadt, Germany) and cell invasion as previously described [18]. In addition, levels of total and phospho-EGFR were determined by ELISA (R&D Systems), according to the manufacturer’s instructions.

**cell lines and reagents**

The following panel of breast cancer cell lines was used: HCC1143, BT20, Hs578t, Hs5788, MDA-MB-231, MDA-MB-468, HCC1937 (all TN), MDA-MB-453, BT474, JIMT-1 and MCF-7 (all non-TN). All were obtained from the American Tissue Culture Collection, apart from Hs5788 which were supplied by Dr Susan McDonnell [19]. This cell line was derived from the parental Hs578t cell line by sequential selection through *in vitro* invasive chambers [19]. All cell lines were maintained in RPMI original articles
1640, supplemented with 10% foetal bovine serum (Invitrogen), 1% penicillin/streptomycin (Invitrogen Life Technologies) and 1% Fungizone (Invitrogen) and maintained in a 37°C CO2 humidified incubator. The specific ADAM-17 inhibitor, PF-5480090, was provided by Pfizer [10, 11]. For brevity, we will refer to this compound as PF-548. Ten millimolar stocks of cisplatin, carboplatin, doxorubicin, 5-fluorouracil (5FU; Sigma Aldrich), gefitinib (SelleckChem, Houston, TX, USA), neratinib and afatinib (Sequoia Research Products, Pangbourne, UK) were prepared and stored according to the manufacturer’s instructions.

**statistical analysis**

Mann–Whitney U tests were utilized when comparing the levels of ADAM-17 protein forms in TN versus non-TN breast cancer specimens. Fisher’s exact test was used to compare categorical ADAM-17 cytoplasmic expression with triple-negative versus non-triple-negative breast cancer specimens. In addition, Student’s paired t test was used to compare the effects of the ADAM-17 inhibitor alone versus combinations of this inhibitor with other agents. Data were analyzed using the PASW Statistics Version 18 (SPSS Inc., Chicago, IL, USA).

**results**

**ADAM-17 expression in basal/TN breast cancers**

Initially, we determined that our target of interest, ADAM-17, was present in TN breast cancer. Using pooled data from 12 published gene expression profiles, ADAM-17 mRNA expression was found to be substantially higher in the basal type than in the other molecular subtypes of breast cancer ($P < 0.001$; Figure 1A). As mentioned above, $\sim$80% of basal-type breast cancers are TN. To confirm these findings at the protein level, ADAM-17 was measured by both western blotting and immunohistochemistry. ADAM-17 protein has previously been shown to exist in two main forms in human breast tissue [16], migrating with molecular masses of $\sim$120 kDa (precursor ADAM-17) and 100 kDa (active ADAM-17). The levels of both these proteins were substantially higher in the TN breast carcinomas than non-TN samples (for 120 kDa, $P = 0.012$; for 100 kDa, $P = 0.045$, Mann–Whitney U test; Figure 1B and C). Using immunohistochemistry, ADAM-17 was mostly located in the cytoplasm (Figure 2A–E). Similar to the western blotting results, high cytoplasmic staining intensity (i.e. samples with an intensity score of 3) was found more frequently in TN than non-TN tumours ($P < 0.001$, Fisher’s test; Figure 2F, supplementary Table S3, available at *Annals of Oncology* online).

**effect of PF-548 on TGF-α shedding**

As ADAM-17 (mRNA and protein) was preferentially expressed in basal/TN breast cancer, we decided to investigate the effects of the ADAM-17 selective inhibitor, PF-548 (Pfizer), on a panel of seven TN breast cancer cell lines *in vitro*. In addition, a panel of four non-TN breast cancer cells were also used as controls. As the release of TGF-α is catalyzed by ADAM-17 [20], we initially investigated the effect of PF-548 on its shedding. The levels of TGF-α released into culture medium were measured by ELISA following 24 h treatment with PF-548. Depending on the cell line, the inhibition of ligand release varied from approximately 5%–67% (Table 1). Most inhibition was obtained using HCC1143, MDA-MB-231, BT474 and JIMT-1 cells, while no substantial inhibition was detected with the Hs578t, Hs578i8 or BT20 cell lines.

**effect of PF-548 on activation of EGFR**

To analyze the effect of ADAM-17 inhibition on downstream EGFR signalling, we assessed the levels of phosphorylated EGFR following treatment with PF-548. Again, depending on the cell line, the decrease in phosphorylated EGFR was variable, ranging effectively from 0 to almost 60% (Table 2). Interestingly, the MDA-MB-231 cells that showed the greatest inhibition of TGF-α release by PF-548, also showed the greatest reduction in phosphorylated EGFR following treatment with the inhibitor. Overall, however, there was no substantial association between PF-548-mediated inhibition of TGF-α release and decreased formation of phosphorylated EGFR. Indeed, the Hs578t cell line which appeared to show no inhibition of TGF-α release exhibited substantially reduced the levels of EGFR phosphorylation. The apparent inability to show TGF-α here may relate to the low baseline levels of TGF-α in this cell line. Consequently, limitations in the sensitivity of the TGF-α ELISA precluded us from seeing reduced levels following PF-548 treatment.

**effect of PF-548 on proliferation of breast cancer cell lines**

Having shown that PF-548 inhibited the release of TGF-α and decreased the formation of phosphorylated EGFR, we next investigated the effect of the ADAM-17 inhibitor on cell proliferation. Treatment with PF-548 resulted in a variable decrease in cell growth, as determined by cell counts (Figure 3A). The greatest decrease was found in the TN cell lines, MDA-MB-468 and Hs578i8 and the HER2-positive cell line, BT474. Overall, response to PF-548 was unrelated to whether cell lines were TN or non-TN.

As ADAM-17 is the primary target for PF-548, we investigated whether levels of its catalytic activity might be associated with the extent of inhibition of proliferation. As seen in Figure 3B, a substantial and moderately strong correlation was found between ADAM-17 activity and the extent of proliferation inhibition ($r = 0.809; P = 0.003; n = 11$). This *in vitro* finding suggests that ADAM-17 activity may be a predictive biomarker of response to PF-548.

To confirm these results observed using monolayer cultures and the MTT growth assay, we also investigated the effects of PF-548 on clonogenic cell growth as it has been previously shown that clonogenic survival is a more sensitive measurement of toxic effect than cellular viability [21]. We treated cells with concentrations of PF-548 ranging from 0.1 to 10 µM and measured their clonogenic ability after 14 days. Compared with vehicle-treated cells, fewer colonies were formed when cells were treated with PF-548 (supplementary Figure S1, available at *Annals of Oncology* online). As with both the MTT assay and cell counting experiments, the magnitude of inhibition of clonogenic growth was cell line dependent.
effect of pre-treatment with PF-548 on sensitivity to chemotherapeutic and EGFR/HER-targeted agents

As ADAM-17, by mediating release of EGFR ligands and stimulating EGFR signalling, was previously reported to confer resistance to different cytotoxic agents [18], we investigated if pre-treatment with PF-548 enhanced response to standard chemotherapeutic agents and EGFR/HER antagonists. Cells were pre-treated with PF-548 for 24 h before the addition of above-mentioned agents. Results are summarized in Figure 4 and (supplementary Figure S2, available at Annals of Oncology online). As can be seen, pre-treatment of cells with the ADAM-17 inhibitor enhanced the anti-proliferative effects of the cytotoxic agents and EGFR/HER antagonists in a cell line and drug-dependent manner. Pre-treatment with PF-548 particularly enhanced response to the pan-HER inhibitor, neratinib in MDA-MB-231 and HCC1143 TN cells. However, depending on the cell line, pre-treatment also increased response to afatinib, gefitinib, carboplatin and doxorubicin (Figure 4 and supplementary Figure S2, available at Annals of Oncology online). As above, we also assessed the clonogenic survival of MDA-MB-231 and HCC1937 TN cells following treatment with neratinib or a combination of neratinib with PF-548. The combination of PF-548 with neratinib resulted in substantially fewer colonies compared with either the inhibitor alone (P < 0.05, Student's t-test versus either the compound alone; supplementary Figure S3, available at Annals of Oncology online).

discussion

As mentioned in the Introduction above, identification and validation of new targets for the treatment of TN breast cancer is urgently needed. In this investigation, we showed that ADAM-17 expression at both mRNA and proteins levels were...
substantially higher in basal/TN breast cancer than the other molecular subtypes. Although ADAM-17 levels were higher in extracts of basal/TN breast tumours than non-TN samples, our preclinical studies showed that specific TN and non-TN breast cancer cell lines investigated were sensitive to ADAM-17 inhibition. A potentially important clinical finding to emerge from our preclinical work was the substantial relationship between ADAM-17 activity and the extent of inhibition by PF-548. If confirmed in further preclinical research, the measurement of ADAM-17 activity could be a potential predictive marker for response to PF-548, assuming this agent was to undergo clinical trials.

Our hypothesis for testing ADAM-17 as a therapeutic target for TN breast cancer was based on the ability of this protease to release most of the ligands that activates EGFR [20, 22]. As mentioned above, EGFR has been implicated in basal/TN breast cancer [4–6, 23]. Indeed, in our study, substantially higher levels of EGFR mRNA were found in the basal-type breast cancers than the other molecular subtypes (supplementary Figure S4, available at Annals of Oncology online). Previously, EGFR signalling was reported to be increased in basal/TN breast cancer cell lines compared with the other types of breast cancer cell lines [4, 5]. All of these studies, when taking together suggest that the inhibition of EGFR signalling may be a new approach for targeting TN breast cancer. Early phase clinical trials with EGFR inhibitors in patients with unselected breast cancer however, have been disappointing [24–26]. One of the major deficiencies in all of these trials was that predictive markers were not used to identify potentially responsive patients. Based on studies in advanced colorectal and non-small-cell lung cancer, we know that the optimum efficacy of EGFR antagonists requires the use of predictive biomarkers [27].

Table 1. Effect of PF-548 on the inhibition of TGF-α release

<table>
<thead>
<tr>
<th>Cell line</th>
<th>TGF-α level (pg/ml)</th>
<th>ADAM17 activity (RFU/mg)</th>
<th>Inhibition (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC1143</td>
<td>78.8 ± 3.3</td>
<td>527.1 ± 20.9</td>
<td>59.0 ± 2.2</td>
<td>0.033</td>
</tr>
<tr>
<td>BT20</td>
<td>30.9 ± 0.3</td>
<td>789.2 ± 3.6</td>
<td>22.8 ± 9.8</td>
<td>0.326</td>
</tr>
<tr>
<td>Hs578t</td>
<td>25.2 ± 1.8</td>
<td>682.7 ± 19.7</td>
<td>4.6 ± 3.6</td>
<td>0.858</td>
</tr>
<tr>
<td>Hs578i8</td>
<td>73.3 ± 0.3</td>
<td>915.1 ± 18.9</td>
<td>20.4 ± 7.7</td>
<td>0.128</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>302.7 ± 39.6</td>
<td>573.1 ± 31.1</td>
<td>66.7 ± 4.0</td>
<td>0.005</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>128.0 ± 0.3</td>
<td>1696.5 ± 7.8</td>
<td>49.4 ± 0.6</td>
<td>0.037</td>
</tr>
<tr>
<td>HCC1937</td>
<td>20.4 ± 0.3</td>
<td>422.3 ± 20.8</td>
<td>20.8 ± 1.4</td>
<td>0.094</td>
</tr>
<tr>
<td>MDA-MB-453</td>
<td>140.3 ± 7.4</td>
<td>474.4 ± 21.6</td>
<td>46.5 ± 2.5</td>
<td>0.014</td>
</tr>
<tr>
<td>BT474</td>
<td>294.5 ± 10.5</td>
<td>719.7 ± 73.7</td>
<td>60.3 ± 5.6</td>
<td>0.009</td>
</tr>
<tr>
<td>JIMT1</td>
<td>422.1 ± 27.1</td>
<td>528.9 ± 17.8</td>
<td>58.3 ± 4.1</td>
<td>0.001</td>
</tr>
<tr>
<td>MCF7</td>
<td>87.9 ± 10.2</td>
<td>963.5 ± 2.4</td>
<td>36.8 ± 4.5</td>
<td>0.047</td>
</tr>
</tbody>
</table>

TGF-α levels and ADAM17 activity were determined at baseline. Data were analyzed using the Student’s paired t-test. Values are means ± SEM.
RFU, relative fluorescence units.

Table 2. Effect of PF-548 on reducing formation of pEGFR (relative to dimethyl sulphoxide vehicle control)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Basal EGFR levels (pg/ml)</th>
<th>Reduction in pEGFR levels (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT20</td>
<td>226.9 ± 23.9</td>
<td>29.5 ± 3.9</td>
<td>0.017</td>
</tr>
<tr>
<td>Hs578t</td>
<td>386.0 ± 28.7</td>
<td>47.1 ± 3.9</td>
<td>0.020</td>
</tr>
<tr>
<td>Hs578i8</td>
<td>100.9 ± 34.4</td>
<td>4.1 ± 0.3</td>
<td>0.040</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>143.8 ± 2.9</td>
<td>57.3 ± 3.6</td>
<td>0.004</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>1161.3 ± 28.5</td>
<td>43.1 ± 6.5</td>
<td>0.022</td>
</tr>
<tr>
<td>MCF7</td>
<td>3.75 ± 1.3</td>
<td>27.5 ± 4.1</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Data were analyzed using the Student’s t-test.

pEGFR, phosphorylated epidermal growth factor.
Figure 3. (A) Barchart illustrating the effect of PF-548 (5 µM) on the inhibition of cell growth in a panel of breast cancer cell lines. Results shown are a mean of three experiments carried out in triplicate ± SEM. Black bars = triple negative breast cancer, grey bars = non-triple negative breast cancer. (B) Bivariate scattergram illustrating the direct positive correlation between ADAM-17 activity per mg protein and percentage growth reduction following treatment with PF-548. Data were analyzed using the Spearman rank test.

Figure 4. Barcharts illustrating the effect of PF-548 (1 µM) alone or in combination with pan-HER inhibitors (neratinib, afatinib) on growth of 4 breast cancer cell lines (A–D), as determined by MTT assay. EGFR inhibitors (gefitinib) and chemotherapeutics (cisplatin, carboplatin, 5-fluorouracil and doxorubicin), were all used at a concentration of 1 µM, doxorubicin at a concentration of 0.05 µM and neratinib at a concentration of 0.5 µM. Additional cell lines are shown in Supplementary Figure S2 available at Annals of Oncology online. *P < 0.05 versus PF-548 alone, Student’s paired t-test.
As well as the use of predictive markers, a further potential approach for enhancing the efficacy of anti-EGFR agents is to use these compounds in combination with other targeted drugs. Indeed, preclinical studies have shown that the combination of EGFR antagonists with inhibitors of Notch [28], Src [17] or MET [29] has resulted in enhanced cytotoxicity. In this report, we therefore investigated the combination of PF-548 with three different anti-EGFR/HER agents, i.e. neratinib, afatinib and gefitinib on cell line growth. Of the three EGFR/HER antagonists investigated in combination with PF-548, the pan-HER inhibitor, neratinib was the most potent and exhibited the widest inhibitory effects. A potential way forward with ADAM-17 inhibition in TN breast cancer is therefore in combination with neratinib. Previously, PF-548 was found to act cooperatively with specific EGFR antagonists in blocking DNA replication in colorectal cancer cells [10].

Using colorectal cancer cells, Kyula et al. [18] previously reported that certain chemotherapeutic agents activated ADAM-17, which resulted in EGFR ligand shedding, EGFR activation and drug resistance. Consistent with this finding, decreased expression of ADAM-17 or inhibition with a dual ADAM-10/17 inhibitor was found to sensitize colorectal cancer cells to 5-FU and oxaliplatin [18]. In agreement with these findings, we also observed that treatment with an ADAM-17 selective inhibitor enhanced response to several different cytotoxic agents in a cell type and drug-dependent manner. The variable responses found with the different cell lines are likely to be a reflection of the heterogeneity that exists in human cancers.

As well as mediating the release of specific EGFR ligands, ADAM-17 has also been shown to release heregulins [30], the ligands that bind to and activate HER3 and HER4. Although largely neglected compared with EGFR and HER2, increasing evidence suggests that HER3 is involved in breast cancer [31]. Furthermore, in addition to releasing several EGFR/HER ligands, ADAM-17 has been shown to activate Notch [32] and release TNFα [33, 34]. Both Notch and TNFα signalling also play key roles in malignant transformation [35–38]. Thus, the inhibition of ADAM-17 has the potential to block several pathways important in cancer formation/progression.

Although this appears to be the first study to investigate a potential anticancer role for an ADAM-17 selective inhibitor in TN breast cancer, previous studies showed that a dual ADAM-10/17 inhibitor, i.e. INCB3619 (Incyte Corporation, Wilmington, DE, USA) blocked cancer cell growth in several different preclinical systems [39–41]. An ADAM-10/17 inhibitor similar to INCB3619, i.e. INCB7839 (Incyte), has undergone clinical trials in HER2-positive advanced breast cancer patients [42, 43]. Preliminary results suggest that this drug is well tolerated [42, 43]. In particular, the musculoskeletal side effects that were previously found with broad-spectrum matrix metalloproteases inhibitors were not observed [39]. Furthermore, there was no evidence of drug induced increases in liver enzymes, bone marrow toxicity or increase in cardiomyopathy. In a recently published abstract [42], the administration of INCB7839 and trastuzumab to 51 patients with advanced HER2-positive breast cancer induced response in 13 of 26 (50%) assessable patients. Our in vitro studies reported here suggest that these dual ADAM-10/17 inhibitors; PF-548 may also have anticancer activity in HER2-positive breast cancers.

In conclusion, our results suggest that the inhibition of ADAM-17, especially in combination with a pan HER antagonist, is a potential new approach not only for the treatment of TN breast cancer but also for other types of breast cancer. Our in vitro studies require confirmation in animal models. Assuming confirmation in vivo, clinical trials focusing on ADAM-17 inhibition, in combination with EGFR/HER-targeted drugs in TN breast cancer may be warranted.

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**Disclosure**

The authors have declared no conflicts of interest.

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