Article

Differential Effects of Afatinib on Cytokine- and Oncology-Related Profiles in Mesenchymal and Basal-Like 1 Triple-Negative Breast Cancer Cells

Deok-Soo Son [1,*], Joan E. Obot [1], Kathleen Campbell [2], Eun-Sook Lee [3], and Samuel E. Adunyah [1]

[1] Department of Biochemistry, Cancer Biology, Neuroscience and Pharmacology, School of Medicine, Meharry Medical College, Nashville, TN 37208, USA
[2] Biology Program, Trevecca Nazarene University, Nashville, TN 37210, USA
[3] Department of Pharmaceutical Sciences, College of Pharmacy, Florida A&M University, Tallahassee, FL 32301, USA
* Correspondence: dson@mmc.edu
Received: 4 July 2025; Revised: 28 Jul 2025; Accepted: 27 August 2025; Published: 4 September 2025

Abstract: Triple-negative breast cancer (TNBC) is an aggressive subtype associated with high recurrence, metastasis, and mortality rates. Elevated epidermal growth factor receptor (EGFR) expression in TNBC cells positions it as a promising target for tyrosine kinase inhibitors (TKIs). This study compared the effects of afatinib on cytokine- and oncology-related profiles in mesenchymal BT549 vs. basal-like 1 MB468 TNBC cell lines. We assessed cytotoxicity, signaling pathways, cytokine- and oncology-related protein expression, and overall survival, using cell viability assay, immunoblots, human proteomic arrays, and TNBC datasets. Afatinib exhibited superior cytotoxicity compared to erlotinib, gefitinib, and lapatinib in both TNBC cells. Afatinib suppressed AKT and ERK activation in both cell lines and reduced vimentin and N-cadherin expression in BT549 cells. As cytokines are involved in TNBC development and progression, afatinib altered cytokine-related profiles, decreasing CD147, FGF2, GDF-15, and MIF, with variable CD71 and uPAR expression in BT549 cells, and reducing CD147 and PDGFB in MB468 cells. Oncology-related profile analysis showed decreased FGF2, GAL3, p53, survivin, and vimentin, and increased CTSD, HSP32, and PGRN in BT549 cells, while capG, GAL3, nectin-4, p53, and survivin were reduced in MB468 cells. Notably, elevated GAL3 and nectin-4 expressions were correlated with worse overall survival in basal-like 1 TNBC patients. In conclusion, afatinib exhibits superior cytotoxicity and selectively modulates cytokine- and oncology-related signatures in TNBC subtypes, warranting further mechanistic and in vivo studies to evaluate its therapeutic potential for TNBC.

Keywords: afatinib; cytokine; overall survival; triple negative breast cancer

1. Introduction

Triple-negative breast cancer (TNBC), comprising 15–20% of breast cancer cases, is the most aggressive subtype, characterized by high rates of metastasis and mortality [1]. Defined by the absence of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2) expression, TNBC is associated with a poor prognosis [1]. TNBC is heterogeneous, including subtypes with distinct molecular profiles: basal-like 1 (enriched in cell cycle pathways), basal-like 2 (growth factor signaling), immunomodulatory (immune cell signaling), mesenchymal and mesenchymal stem-like (epithelial-mesenchymal transition), and luminal androgen receptor (androgen receptor signaling) [2]. Understanding these molecular and clinical differences may guide the development of targeted therapies to improve survival outcomes.

Our previous study revealed elevated epidermal growth factor receptor (EGFR) expression and increased proinflammatory chemokine production in TNBC cells compared to non-TNBC cells [3]. These chemokines, regulated by EGFR-mediated signaling, likely exacerbate inflammatory burden in the TNBC tumor microenvironment [4]. Tyrosine kinase inhibitors (TKIs) targeting EGFR signaling may thus offer therapeutic potential for TNBC. However, variable responses to EGFR-targeted therapies and resistance highlight the need for personalized treatment strategies [5]. Notably, the basal-like TNBC subtype exhibits higher EGFR expression than other subtypes [3]. Therefore, we selected basal-like and mesenchymal TNBC cell lines to evaluate the

cytotoxicity of TKIs, including afatinib, lapatinib, gefitinib, and erlotinib. Afatinib demonstrated superior efficacy among the tested TKIs. Consequently, we investigated afatinib's effects on EGFR-mediated signaling, epithelial-mesenchymal transition (EMT), cytokine profiles, and oncology-related proteins, alongside analyzing overall survival for afatinib-regulated genes in basal-like and mesenchymal TNBC patients.

2. Materials and Methods

2.1. TNBC Cell Lines and Culture

TNBC cells exhibit significantly higher EGFR protein expression compared to non-TNBC cells [3]. BT549 and MB468 TNBC cell lines (American Type Culture Collection, Manassas, VA, USA) were chosen as models for human mesenchymal and basal-like 1 TNBC subtypes, respectively, due to their elevated EGFR expression levels [3]. Cell lines were routinely tested for mycoplasma contamination using the Lonza MycoAlert Kit (Lonza, Allendale, NJ, USA), confirming negative results. Cells were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin at 37 °C in a humidified incubator with 5% CO_2 and 95% air. All liquid culture media were purchased from Invitrogen (Grand Island, NY, USA). TKIs, such as afatinib, lapatinib, gefitinib, and erlotinib, were purchased from Cayman Chemical (Ann Arbor, MI, USA).

2.2. Cell Viability Assay

Cell viability was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, as previously described [6]. After a 48-h incubation in 24-well plates, cells were washed twice with phosphate-buffered saline (PBS). MTT solution (1 mg/mL in phenol red-free media: PBS = 4:1) was added, and the plates were incubated for 3 h, protected from light. The MTT solution was removed, and 500 µL of isopropanol was added to dissolve the formazan product. Plates were agitated for 10 min at room temperature, and optical density was measured at 595 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). Results were normalized to the untreated control group.

2.3. Western Blot Analysis

Whole-cell lysates were prepared, separated by SDS-polyacrylamide gels, and transferred to nitrocellulose membranes following established procedures [6]. Primary antibodies against AKT, ERK, SAPK/JNK, and p38 and their phosphorylated forms, E-cadherin, N-cadherin, vimentin, PCNA, and p21 (Cell Signaling Technology, Beverly, MA, USA) were used. β-actin (TU-02, Santa Cruz Biotechnology, Dallas, TX, USA) served as the loading control. Protein bands were detected using chemiluminescence kits (MilliporeSigma, St. Louis, MO, USA) and quantified with ImageJ software (https://imagej.net; version: ImageJ 1.54P)

2.4. Proteomic Array Analysis

Cytokine and oncology-related protein profiles were evaluated using the Proteome Profiler Human XL Cytokine Array (ARY022B) and Oncology Array (ARY026) (R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions as described previously [6]. Spot intensities were quantified with ImageJ (https://imagej.net) by subtracting background signals and normalizing to reference spots.

2.5. Overall Survival Analysis

Overall survival was evaluated using the Kaplan-Meier Plotter database (https://kmplot.com/analysis/index.php?p=service&cancer=breast, accessed on 11 March 2025). Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated via proportional hazards regression based on the gene expression profile from 655 ovarian cancer patients in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) database [7].

2.6. Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical significance ($p < 0.05$) was determined using Student's *t*-test or one-way analysis of variance (ANOVA). Significant ANOVA results were followed by Tukey's post-hoc comparisons.

3. Results

3.1. Afatinib Exhibits Superior Cytotoxicity in Mesenchymal and Basal-Like 1 TNBC Cells

To evaluate the cytotoxic effects of TKIs, including erlotinib, gefitinib, lapatinib, and afatinib, we used mesenchymal (BT549) and basal-like 1 (MB468) TNBC cell lines. Cell viability was assessed using the MTT assay, and the half-maximal inhibitory concentrations (IC50) were determined as follows: 382.2 μM erlotinib, 46.8 μM gefitinib, 21.6 μM lapatinib, and 5.8 μM afatinib for BT549 cells; 168.5 μM erlotinib, 31.4 μM gefitinib, 4.1 μM lapatinib, and 1.6 μM afatinib for MB468 cells (Figure 1). In both cell lines, the potency order was afatinib > lapatinib > gefitinib > erlotinib. Afatinib demonstrated superior cytotoxicity, requiring lower concentrations to inhibit TNBC cell viability compared to other TKIs. Notably, all TKIs were more effective against MB468 cells than BT549 cells (Figure 1).

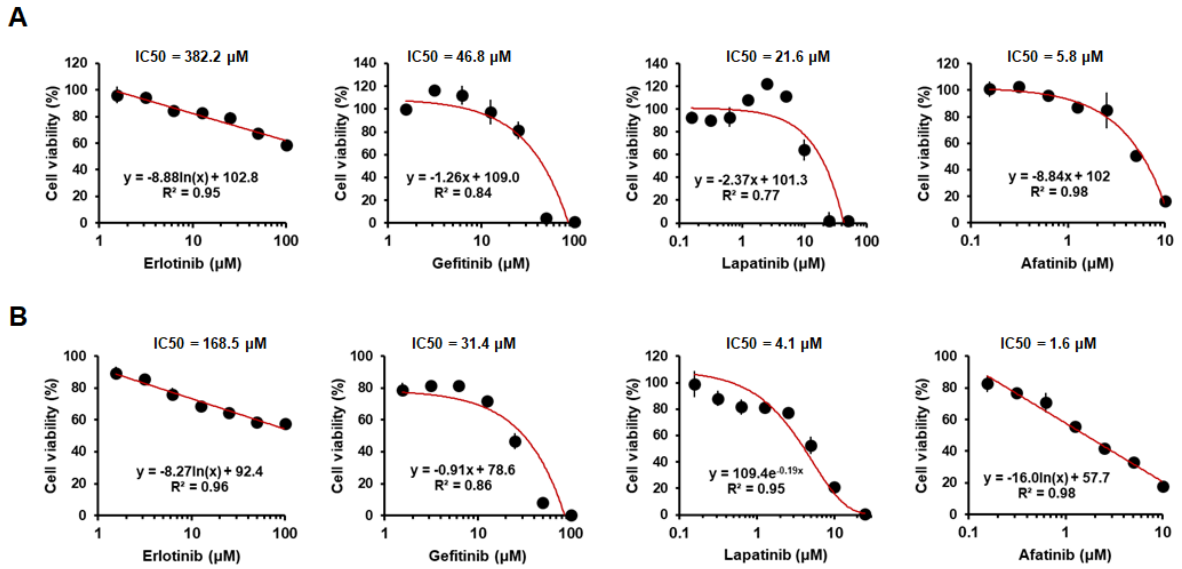

Figure 1. Comparisons of IC50 values for tyrosine kinase inhibitors in mesenchymal vs. basal-like 1 TNBC cells. (**A**) Effects of TKIs (erlotinib, gefitinib, lapatinib, afatinib) on cell viability in mesenchymal BT549 TNBC cells. (**B**) Effects of TKIs on cell viability in basal-like 1 MB468 TNBC cells. Cells were treated with various TKI concentrations for 48 h. Data points represent mean values, with vertical bars indicating SD (n = 3). Red trendlines were generated using exponential, linear, and logarithmic regression, selected for the highest R^2 values, calculated with Microsoft Excel's Data Analysis Tools.

3.2. Afatinib Suppresses AKT and ERK Activation and Downregulates EMT Proteins

Based on the comparative cytotoxicity of TKIs (Figure 1), afatinib was selected to evaluate its effects on signaling pathways in BT549 and MB468 cells. Afatinib significantly reduced AKT activation in BT549 cells and ERK activation in MB468 cells, with no notable impact on SAPK/JNK or p38 activation in either cell line (Figure 2A). Additionally, afatinib downregulated EMT proteins, including vimentin and N-cadherin, in BT549 cells (Figure 2B). No significant changes were observed in the proliferative marker PCNA or the cell cycle inhibitor p21 in either cell line (Figure 2B). These findings indicate that afatinib exerts its mechanistic effects by inhibiting PI3K/AKT and ERK signaling in mesenchymal and basal-like 1 TNBC cells, while particularly downregulating EMT-related proteins in mesenchymal TNBC, thereby reducing cell proliferation and migration.

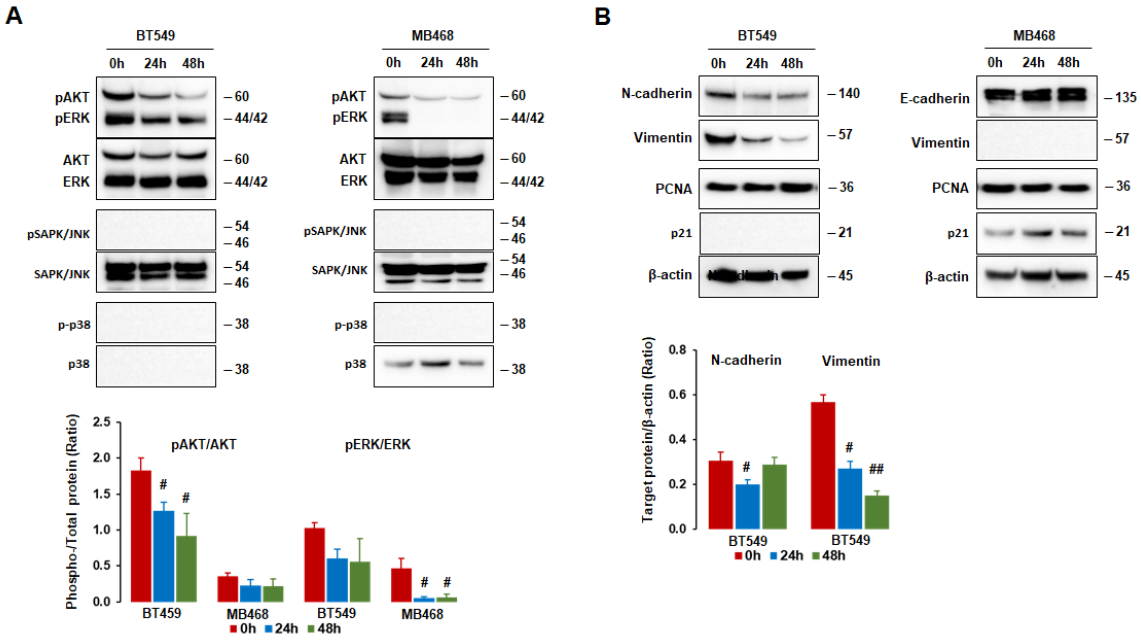

Figure 2. Afatinib-induced regulation of signaling pathways and EMT proteins in BT549 and MB468 cells. (**A**) Effects of afatinib on the activation of AKT, ERK, SAPK/JNK, and p38 in BT549 and MB468 cells. (**B**) Effects of afatinib on EMT-associated proteins (vimentin, N-cadherin), PCNA, and p21 in both cell lines. Cells were treated with afatinib at its IC50 concentration for 24 h and 48 h. # and ## denote statistical significance ($p < 0.05$, n = 3) between groups, as determined by ANOVA.

3.3. Afatinib Alters Specific Cytokine-Related Profiles in Mesenchymal and Basal-Like 1 TNBC Cells

Afatinib treatment modified cytokine profiles in BT549 and MB468 cells. In BT549 cells, afatinib reduced levels of CD147, FGF2, GDF-15, and MIF, with CD71 and uPAR showing upregulation at 24 h and downregulation at 48 h (Figure 3). In MB468 cells, afatinib decreased CD147 and PDGFB levels (Figure 3). Notably, CD147 was consistently reduced in both cell lines. Overall, MB468 cells exhibited lower baseline cytokine expression compared to BT549 cells.

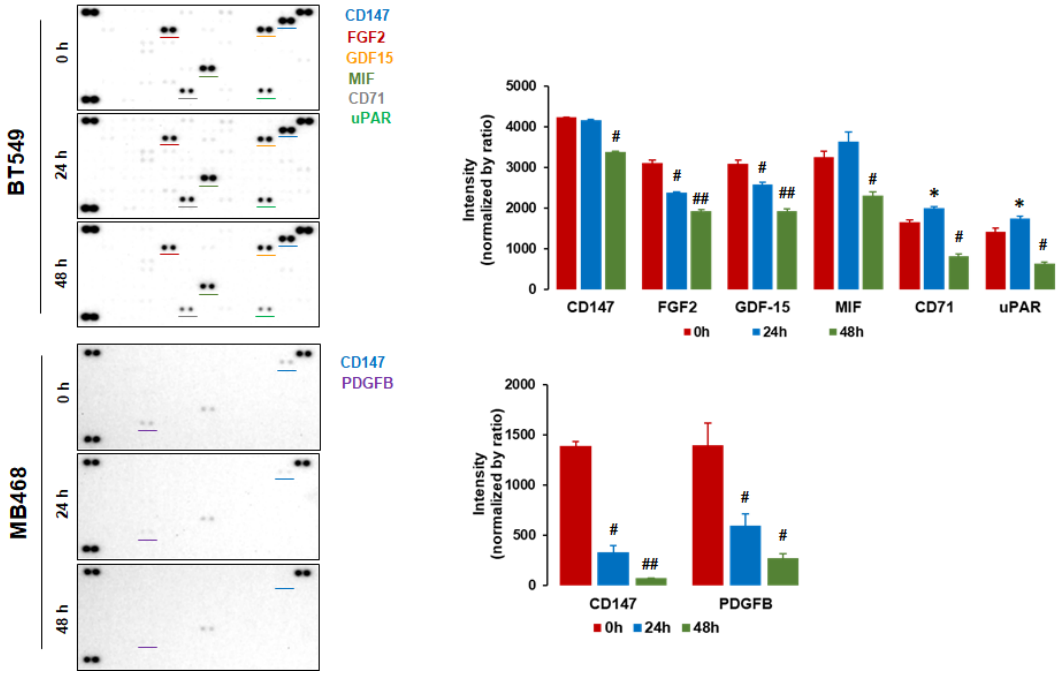

Figure 3. Effects of afatinib on cytokine-related profiles in BT549 and MB468 TNBC cells. Cells were treated with afatinib at their respective IC50 concentration for 24 and 48 h. Cytokine expression levels were assessed, revealing significant changes. Statistical significance was determined by ANOVA (*, #, and ## indicate $p < 0.05$,

n = 2) for comparisons between groups. CD147, cluster of differentiation 147; FGF2, fibroblast growth factor 2; GDF-15, growth differentiation factor 15; MIF, macrophage migration inhibitory factor; CD71, cluster of differentiation 71; uPAR, urokinase plasminogen activator surface receptor; PDGFB, platelet-derived growth factor subunit B.

3.4. Afatinib Differentially Modulates Oncology-Related Proteins in Mesenchymal and Basal-Like 1 TNBC Cells

The oncology array revealed that afatinib treatment distinctly altered protein expression in BT549 and MB468 TNBC cells. In BT549 cells, afatinib downregulated FGF2, GAL3, p53, survivin, and vimentin, while upregulating CTSD, HSP32, and PGRN. In MB468 cells, afatinib reduced the expression of CapG, GAL3, nectin-4, p53, and survivin (Figure 4). Notably, GAL3, p53, and survivin were consistently downregulated in both cell lines in response to afatinib.

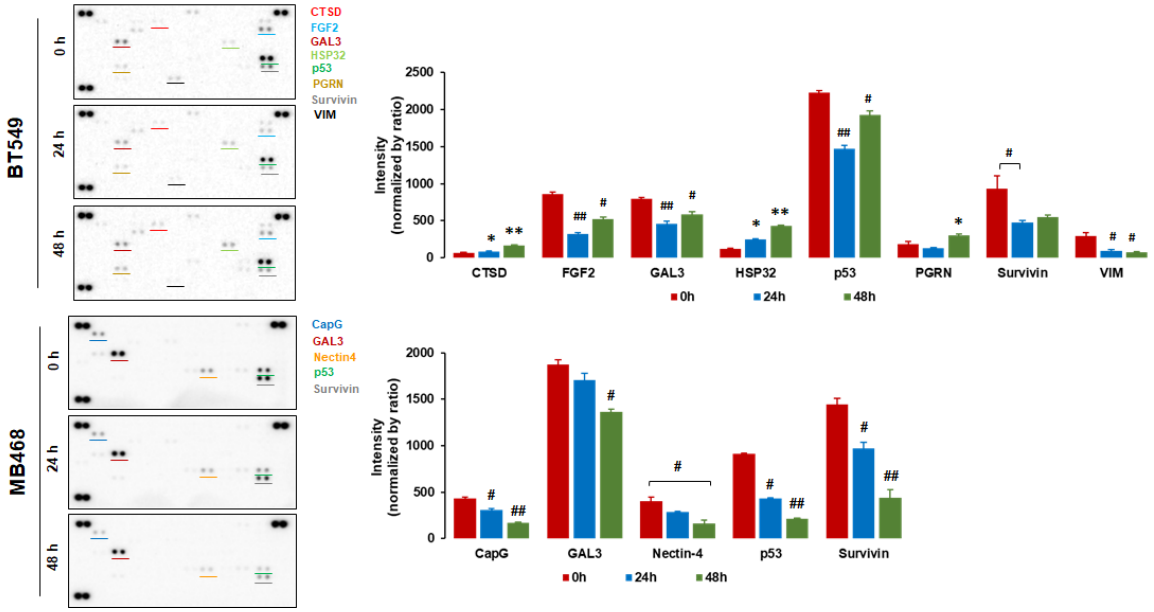

Figure 4. Effects of afatinib on oncology-related protein profiles in BT549 and MB468 TNBC cells. Cells were treated with afatinib at their respective IC50 concentrations for 24 and 48 h. Protein expression levels were evaluated, revealing significant changes. Statistical significance was determined by ANOVA (*, **, #, and ## indicate $p < 0.05$, n = 2) for comparisons between groups. CTSD, cathepsin D; FGF2, fibroblast growth factor 2; GAL3, galectin-3; HSP32, heat shock protein 32; PGRN, progranulin; VIM, vimentin; CapG, capping actin protein, gelsolin like.

3.5. Afatinib-Regulated Genes Influence Overall Survival in Mesenchymal vs. Basal-Like 1 TNBC Patients

We analyzed the relationship between afatinib-regulated cytokines and oncology-related proteins (Figures 3 and 4) and overall survival in patients with mesenchymal and basal-like 1 TNBC. Statistical analysis identified significant associations with overall survival for FGF2, survivin, and CD71 in mesenchymal TNBC patients, and for nectin-4 and GAL3 in basal-like 1 TNBC patients. Higher expression of FGF2, survivin, and CD71 was associated with improved overall survival in mesenchymal TNBC patients (Figure 5A). Conversely, elevated nectin-4 and GAL3 expressions were associated with worse overall survival in basal-like 1 TNBC patients (Figure 5B). These results indicate that afatinib may enhance overall survival in basal-like 1 TNBC patients by downregulating nectin-4 and GAL3 expressions.

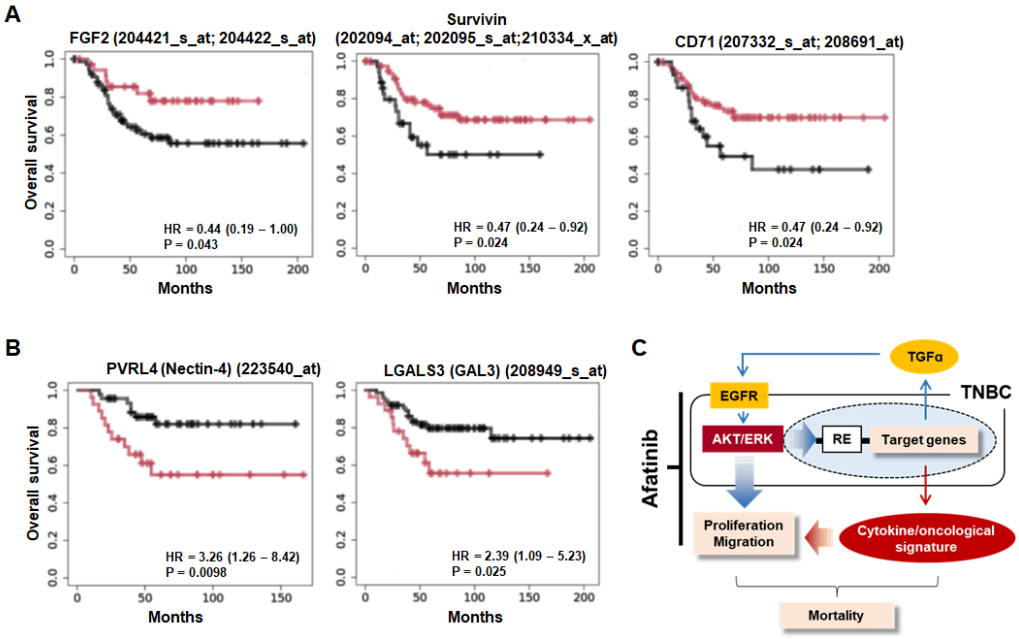

Figure 5. Overall survival of afatinib-regulated genes in mesenchymal vs. basal-like 1 TNBC patients. (**A**) Overall survival of mesenchymal TNBC patients on FGF2, surviving, and CD71 expressions (n = 114). (**B**) Overall survival of basal-like 1 TNBC patients on nectin-4 and GAL3 expressions (n = 103). HRs (95% CIs) for overall survival of TNBC patients are indicated on the plots. The HRs (95% CIs) were determined using the GEO and TCGA datasets available in the Kaplan-Meier plotter database (https://kmplot.com/analysis/, accessed on 12 March 2025). Black and red lines indicate low and high levels of targeted genes, respectively. (**C**) Diagram for the molecular mechanisms of afatinib to inhibit AKT and ERK activation, as well as cytokine and oncology-related proteins in TNBC cells. Blue arrows represent autocrine and paracrine TGFα-EGFR-AKT/ERK signaling, and red arrows represent inflammatory burden. RE: responsive elements. In TNBC, elevated EGFR and TGFα activate AKT and ERK signaling via the TGFα–EGFR axis, promoting proliferation, migration, and cancer progression. Autocrine and paracrine activation further elevates cytokines and oncogenic proteins, driving inflammation and mortality. Afatinib blocks AKT and ERK signaling, suppresses EMT-driven migration, and reduces oncogenic cytokines, thereby slowing progression and improving survival.

4. Discussion

This study demonstrated superior cytotoxicity of afatinib through suppression of AKT and ERK signaling pathways, inhibition of EMT, differential regulation of cytokine- and oncology-related protein signatures in mesenchymal and basal-like 1 TNBC subtypes. Data from the Genomics of Drug Sensitivity in Cancer database (https://www.cancerrxgene.org/, accessed on 28 June 2025) revealed the following IC50 values for TKIs in TNBC cell lines: 6.28 μM in MB468 and 81.2 μM in BT549 for erlotinib; 6.23 μM in MB468 and 94.6 μM in BT549 for gefitinib; 3.70 μM in MB468 and 24.3 μM in BT549 for lapatinib; 1.19 μM in MB468 and 7.67 μM in BT549 for afatinib. These results confirmed the superior cytotoxicity of afatinib compared to other TKIs, consistent with our findings (Figure 1). The heightened sensitivity of MB468 cells to afatinib, compared to BT549 cells, may be attributed to elevated EGFR expression in MB468 cells, as reported in our previous study [3]. In addition, The mean IC50 values for afatinib across cell lines from major cancer types are as follows: 1.55 μM in esophageal cancer, 1.58 μM in head and neck cancer, 3.13 μM in lung squamous cancer, 3.34 μM in lung adenocarcinoma, 3.75 μM in stomach cancer, 3.87 μM in breast cancer, 3.93 μM in colorectal cancer, 4.98 μM in renal cancer, 5.11 μM in neuroblastoma, 5.38 μM in ovarian cancer, 6.06 μM in small cell lung cancer, 7.98 μM in bladder cancer, 9.23 μM in uterine cancer, 11.1 μM in prostate cancer, 11.4 μM in pancreatic cancer, 11.5 μM in skin cancer, 12.6 μM in thyroid cancer, 12.7 μM in glioblastoma, 13.0 μM in liver cancer (https://www.cancerrxgene.org/, accessed on 15 August 2025). These data suggest that afatinib's efficacy varies significantly across different cancer types.

Afatinib, as a TKI, significantly reduced AKT and ERK activation in TNBC cells (Figure 2A) and downregulated EMT markers, including vimentin and N-cadherin, specifically in mesenchymal TNBC cells (Figure 2B), aligning with findings in gastric adenocarcinoma cells [8]. Notably, no significant changes in the proliferative marker PCNA or the cell cycle inhibitor p21 were observed (Figure 2B), suggesting that afatinib influences cell viability and survival through AKT and ERK pathways primarily rather than pathways related to proliferation or cell cycle regulation.

Afatinib also reduced CD147 expression in TNBC cells (Figure 3), consistent with observations in non-small cell lung cancer stem cells [9]. Additionally, afatinib-downregulated FGF2 expression may mitigate survival mechanisms in TNBC cells, as FGF2 upregulation is associated with afatinib resistance in non-small cell lung cancer cells [10]. Afatinib's effect on p53 expression varied by cell type: it reduced p53 in TNBC cells (Figure 4) and in tumors from retinoblastoma-transplanted mice [11], but showed no effect in non-small cell lung cancer cells [12]. This variability likely reflects cell-specific responses to afatinib. Similarly, afatinib-downregulated survivin in TNBC cells (Figure 4) corroborates findings of gefitinib-reduced survivin in PC-9 brain-seeking cells [13]. Western blot analyses with afatinib's suppression of vimentin (Figure 2) are consistent with array data (Figure 4). Afatinib also modulated expression levels of GDF-15, MIF, CD71, uPAR, PDGFB, GAL3, CTSD, HSP32, CapG, and nectin-4 (Figures 3 and 4), though further studies are needed to elucidate the mechanisms underlying these changes.

Among afatinib-regulated proteins, elevated expression of FGF2, survivin, and CD71 was associated with improved overall survival in mesenchymal TNBC patients (Figure 5A). This finding suggests a paradoxical effect, as afatinib's downregulation of these proteins does not appear to drive survival benefits in this TNBC subtype. Additionally, afatinib's efficacy in tumor tissues may vary due to interactions with other cell types in the tumor microenvironment, in contrast to cell models lacking such interactions. Conversely, increased nectin-4 and GAL3 expressions was associated with worse overall survival in basal-like 1 TNBC patients (Figure 5B). Afatinib's downregulation of these proteins (Figure 4) suggests it may improve survival more effectively in basal-like 1 TNBC patients than in mesenchymal TNBC patients. However, while afatinib reduces survivin expression in MB468 cells (Figure 4), survivin-associated survival was not observed in basal-like 1 TNBC patients (data not shown), in contrast to mesenchymal TNBC patients. This is further supported by lower IC50 values in basal-like 1 TNBC cells compared to mesenchymal TNBC cells (Figure 1), indicating afatinib's greater effects in the basal-like 1 subtype.

Despite afatinib's potent cytotoxicity (Figure 1), clinical trials of EGFR inhibitors in breast cancer have yielded low response rates [14]. Overexpression of insulin-like growth factor binding protein 2 (IGFBP2) is implicated in afatinib resistance in TNBC cells [5], highlighting potential resistance mechanisms in TNBC patients. However, TNBC patients with amplified DAXX gene exhibited favorable responses to afatinib [15], suggesting that TNBC's molecular heterogeneity influences afatinib efficacy. Afatinib as a standalone treatment showed limited activity in TNBC [16], but its sensitivity was associated with a basal-like phenotype with high pSrc and pMAPK levels [17]. Combining afatinib with dasatinib demonstrated potential clinical benefits in TNBC [17], and combining afatinib with a palmitoylation inhibitor significantly reduced tumor growth and metastasis in 4T1 tumor-bearing mice, prolonging survival [18]. These findings suggest that combining afatinib with targeted therapies may overcome resistance and enhance its efficacy in TNBC.

Unlike non-TNBC cells, TNBC cells exhibit high EGFR and TGFα expression, activating AKT and ERK signaling via the TGFα-EGFR axis [3]. This axis promotes cell proliferation and migration (Figure 5C, blue lines), driving cancer progression. Additionally, autocrine and paracrine activation of the TGFα-EGFR axis enriches cytokine- and oncology-related protein expression (Figure 5C, red lines), increasing inflammatory burden and mortality. Afatinib attenuates AKT and ERK activation, inhibits EMT-related migration, and downregulates oncology-related cytokines and proteins, collectively reducing proliferation and migration followed by improved survival in TNBC (Figure 5C).

In conclusion, afatinib inhibits TNBC progression by suppressing AKT and ERK signaling, EMT, and oncology-related protein expressions, thereby enhancing overall survival, particularly in basal-like 1 TNBC patients. Combining afatinib with other targeted therapies holds promise for overcoming resistance and maximizing therapeutic efficacy in TNBC.

Author Contributions: Conceptualization, D.-S.S.; methodology, D.-S.S., J.E.O. and K.C.; software, D.-S.S., J.E.O. and K.C.; validation, D.-S.S., J.E.O., K.C., E.-S.L. and S.E.A.; formal analysis, D.-S.S., J.E.O. and K.C.; investigation, D.-S.S., J.E.O., and K.C.; resources, D.-S.S., E.-S.L. and S.E.A.; data curation, D.-S.S., J.E.O., and K.C.; writing—original draft preparation, D.-S.S.; writing—review and editing, D.-S.S., J.E.O., K.C., E.-S.L. and S.E.A.; visualization, D.-S.S., J.E.O. and K.C.; supervision, D.-S.S.; project administration, D.-S.S.; funding acquisition, D.-S.S., E.-S.L. and S.E.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded, either in whole or in part, by the National Institutes of Health (NIH) through the following grants: R25CA214220 (K.C.), R01ES031282 (E.-S.L.), SC1CA200519 (D.-S.S.), U54MD007586 (S.E.A.), U54CA163069 (J.E.O., D.-S.S., S.E.A.), and ACS DICRIDG-21-071-01-DICRIDG (D.-S.S., S.E.A.).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Kaplan-Meier plotter database (https://kmplot.com/analysis/, accessed on 11 March 2025) is available based on gene expression profile of breast cancer patients from Biotechnology Information (NCBI) Gene Expression

Omnibus (GEO) database and The Cancer Genome Atlas (TCGA). Publicly available IC50 data are deposited in Genomics of Drug Sensitivity in Cancer (https://www.cancerrxgene.org/, accessed on 28 June and 15 August 2025)

Conflicts of Interest: The authors declare no conflicts of interest with the contents of this article.

References

1. Dogra, A.K.; Prakash, A.; Gupta, S.; Gupta, M. Prognostic Significance and Molecular Classification of Triple Negative Breast Cancer: A Systematic Review. *Eur. J. Breast Health* **2025**, *21*, 101. https://doi.org/10.4274/ejbh.galenos.2025.2024-10-2.
2. Lehmann, B.D.; Bauer, J.A.; Chen, X.; Sanders, M.E.; Chakravarthy, A.B.; Shyr, Y.; Pietenpol, J.A. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J. Clin. Investig.* **2011**, *121*, 2750–2767. https://doi.org/10.1172/jci45014.
3. Ignacio, R.M.C.; Gibbs, C.R.; Lee, E.S.; Son, D.S. The TGFα-EGFR-Akt signaling axis plays a role in enhancing proinflammatory chemokines in triple-negative breast cancer cells. *Oncotarget* **2018**, *9*, 29286–29303. https://doi.org/10.18632/oncotarget.25389.
4. Son, D.S.; Kabir, S.M.; Dong, Y.; Lee, E.; Adunyah, S.E. Characteristics of chemokine signatures elicited by EGF and TNF in ovarian cancer cells. *J. Inflamm.* **2013**, *10*, 25. https://doi.org/10.1186/1476-9255-10-25.
5. Pellecchia, S.; Franchini, M.; Viscido, G.; Arnese, R.; Gambardella, G. Single cell lineage tracing reveals clonal dynamics of anti-EGFR therapy resistance in triple negative breast cancer. *Genome Med.* **2024**, *16*, 55. https://doi.org/10.1186/s13073-024-01327-2.
6. Son, D.S.; Done, K.A.; Son, J.; Izban, M.G.; Virgous, C.; Lee, E.S.; Adunyah, S.E. Intermittent Fasting Attenuates Obesity-Induced Triple-Negative Breast Cancer Progression by Disrupting Cell Cycle, Epithelial-Mesenchymal Transition, Immune Contexture, and Proinflammatory Signature. *Nutrients* **2024**, *16*, 2101. https://doi.org/10.3390/nu16132101.
7. Győrffy, B. Integrated analysis of public datasets for the discovery and validation of survival-associated genes in solid tumors. *Innovation* **2024**, *5*, 100625. https://doi.org/10.1016/j.xinn.2024.100625.
8. Lo, Y.L.; Wang, T.Y.; Chen, C.J.; Chang, Y.H.; Lin, A.M. Two-in-One Nanoparticle Formulation to Deliver a Tyrosine Kinase Inhibitor and microRNA for Targeting Metabolic Reprogramming and Mitochondrial Dysfunction in Gastric Cancer. *Pharmaceutics* **2022**, *14*, 1759. https://doi.org/10.3390/pharmaceutics14091759.
9. Han, J.M.; Kim, S.M.; Kim, H.L.; Cho, H.J.; Jung, H.J. Natural Cyclophilin A Inhibitors Suppress the Growth of Cancer Stem Cells in Non-Small Cell Lung Cancer by Disrupting Crosstalk between CypA/CD147 and EGFR. *Int. J. Mol. Sci.* **2023**, *24*, 9437. https://doi.org/10.3390/ijms24119437.
10. Azuma, K.; Kawahara, A.; Sonoda, K.; Nakashima, K.; Tashiro, K.; Watari, K.; Izumi, H.; Kage, M.; Kuwano, M.; Ono, M.; et al. FGFR1 activation is an escape mechanism in human lung cancer cells resistant to afatinib, a pan-EGFR family kinase inhibitor. *Oncotarget* **2014**, *5*, 5908–5919. https://doi.org/10.18632/oncotarget.1866.
11. Zhan, W.J.; Zhu, J.F.; Wang, L.M. Inhibition of proliferation and induction of apoptosis in RB116 retinoblastoma cells by afatinib treatment. *Tumour Biol.* **2016**, *37*, 9249–9254. https://doi.org/10.1007/s13277-015-4768-1.
12. Liu, Z.; Fu, Q.; Wang, Y.; Cui, L.; Zhang, W.; Teng, Y.; Yu, P. Synergy between vinorelbine and afatinib in the inhibition of non-small cell lung cancer progression by EGFR and p53 signaling pathways. *Biomed. Pharmacother.* **2021**, *134*, 111144. https://doi.org/10.1016/j.biopha.2020.111144.
13. Liu, Z.; Shah, N.; Marshall, K.L.; Sprowls, S.A.; Saralkar, P.; Mohammad, A.; Blethen, K.E.; Arsiwala, T.A.; Fladeland, R.; Lockman, P.R.; et al. Overcoming the acquired resistance to gefitinib in lung cancer brain metastasis in vitro and in vivo. *Arch. Toxicol.* **2021**, *95*, 3575–3587. https://doi.org/10.1007/s00204-021-03147-4.
14. Nakai, K.; Hung, M.C.; Yamaguchi, H. A perspective on anti-EGFR therapies targeting triple-negative breast cancer. *Am. J. Cancer Res.* **2016**, *6*, 1609–1623.
15. Lin, P.H.; Tseng, L.M.; Lee, Y.H.; Chen, S.T.; Yeh, D.C.; Dai, M.S.; Liu, L.C.; Wang, M.Y.; Lo, C.; Chang, S.; et al. Neoadjuvant afatinib with paclitaxel for triple-negative breast cancer and the molecular characteristics in responders and non-responders. *J. Formos. Med. Assoc.* **2022**, *121*, 2538–2547. https://doi.org/10.1016/j.jfma.2022.05.015.
16. Schuler, M.; Awada, A.; Harter, P.; Canon, J.L.; Possinger, K.; Schmidt, M.; De Grève, J.; Neven, P.; Dirix, L.; Jonat, W.; et al. A phase II trial to assess efficacy and safety of afatinib in extensively pretreated patients with HER2-negative metastatic breast cancer. *Breast Cancer Res. Treat.* **2012**, *134*, 1149–1159. https://doi.org/10.1007/s10549-012-2126-1.
17. Canonici, A.; Browne, A.L.; Ibrahim, M.F.K.; Fanning, K.P.; Roche, S.; Conlon, N.T.; O'Neill, F.; Meiller, J.; Cremona, M.; Morgan, C.; et al. Combined targeting EGFR and SRC as a potential novel therapeutic approach for the treatment of triple negative breast cancer. *Ther. Adv. Med. Oncol.* **2020**, *12*. https://doi.org/10.1177/1758835919897546.
18. Wang, X.; Zhu, X.; Li, B.; Wei, X.; Chen, Y.; Zhang, Y.; Wang, Y.; Zhang, W.; Liu, S.; Liu, Z.; et al. Intelligent Biomimetic Nanoplatform for Systemic Treatment of Metastatic Triple-Negative Breast Cancer via Enhanced EGFR-Targeted Therapy and Immunotherapy. *ACS Appl. Mater. Interfaces* **2022**, *14*, 23152–23163. https://doi.org/10.1021/acsami.2c02925.