In Vivo Insights into EGFR-Targeted Breast Cancer Therapy: From Mechanism to Efficacy

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Abstract:

Background Breast cancer is a heterogeneous malignancy with epidermal growth factor receptor (EGFR) overexpression implicated in up to 70% of cases, particularly triple-negative subtypes, driving proliferation, invasion, and angiogenesis through downstream AKT, MAPK, and VEGF pathways. While molecular studies underscore EGFR as a viable target, discrepancies in preclinical validation have hindered clinical translation of inhibitors like gefitinib.

Objective This study translates molecular mechanisms of EGFR signalling into in vivo efficacy assessments using complementary preclinical models, evaluating tumour regression, biomarker modulation, and histopathological changes augmented by artificial intelligence (AI)-based analysis.

Methods Athymic nude mice bearing MDA-MB-468 xenografts and Sprague-Dawley rats with 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumours (n=10/group) were treated with gefitinib (50 mg/kg, oral, 4 weeks) or vehicle. Tumor volumes were measured biweekly via calipers. Post-treatment tissues underwent immunohistochemistry (IHC) and Western blot for phosphorylated AKT (p-AKT), MAPK (p-MAPK), and VEGF. ImageXpert AI software quantified necrosis, proliferation (Ki-67), and biomarker intensity from H&E and IHC slides, validated against manual pathologist scoring.

Results Gefitinib induced significant tumor regression: 62% volume reduction in xenografts ($p<0.001$ vs. control) and 48% in DMBA models ($p<0.01$). Pathway inhibition was evident with 68% decrease in p-AKT, 52% in p-MAPK, and 42% in VEGF expression (all $p<0.05$). AI analysis demonstrated high concordance with manual reads (Pearson's $r=0.91$ for necrosis; $r=0.88$ for Ki-67), identifying 35% greater necrosis in treated groups and subtype-specific vascular remodelling.

Conclusions EGFR-targeted therapy demonstrates robust in vivo efficacy across models, with downstream suppression correlating to histopathological improvements. Integration of ImageXpert enhances quantitative precision, bridging preclinical gaps toward biomarker-stratified clinical trials. These insights advocate for AI-augmented pipelines in oncology drug development.

Keywords: EGFR inhibition; breast cancer models; xenograft tumors; DMBA induction; AKT/MAPK signaling; VEGF expression; AI histopathology; ImageXpert

Introduction:

Breast cancer remains the most common malignancy among women worldwide, accounting for approximately 2.3 million new cases and 685,000 deaths annually as of 2025. Despite advances in screening and multimodal therapies, metastatic disease and therapeutic resistance pose persistent challenges, particularly in aggressive subtypes like triple-negative breast cancer (TNBC), which lacks targeted options beyond chemotherapy. Central to these hurdles is the epidermal growth factor receptor (EGFR, also known as ErbB1), a tyrosine kinase receptor overexpressed in 30–60% of breast tumors, correlating with poor prognosis, increased metastasis, and reduced survival rates.[1]

EGFR activation initiates a cascade of intracellular signaling that promotes oncogenesis. Upon ligand binding (e.g., EGF), EGFR dimerizes and autophosphorylates, recruiting adapters like Grb2 and Shc to activate the Ras-Raf-MEK-ERK (MAPK) pathway, fostering cell proliferation and survival. Concurrently, the PI3K-AKT-mTOR axis is engaged, enhancing anti-apoptotic signals and metabolic reprogramming. Furthermore, EGFR crosstalk with

vascular endothelial growth factor (VEGF) signaling drives angiogenesis, a hallmark of tumor progression, via hypoxia-inducible factor-1α (HIF-1α) upregulation. In breast cancer, this interplay is exacerbated in EGFR-high models, where pathway hyperactivation confers resistance to endocrine and HER2-directed therapies. Recent preclinical insights reveal that EGFR mutations or amplifications occur in 10–15% of cases, further amplifying these effects and underscoring the rationale for targeted inhibition.[2]

The promise of EGFR tyrosine kinase inhibitors (TKIs) like gefitinib and erlotinib, alongside monoclonal antibodies such as cetuximab, has been tempered by mixed clinical outcomes. Phase II/III trials in advanced breast cancer reported modest response rates (8–20%), attributed to heterogeneous EGFR expression, acquired resistance via bypass pathways (e.g., MET or AXL upregulation), and inadequate patient stratification. A 2025 review highlights resistance mechanisms including EGFR nuclear translocation, which promotes DNA repair and evasion of apoptosis, emphasizing the need for robust in vivo validation to refine therapeutic windows. Preclinical models thus serve as critical bridges, recapitulating human tumor biology while enabling mechanistic dissection.[3]

Two paradigmatic models dominate EGFR breast cancer research: orthotopic xenografts and chemically induced autochthonous tumors. Xenograft models, utilizing human cell lines like MDA-MB-468 (EGFR-overexpressing TNBC), implanted subcutaneously or orthotopically in immunodeficient mice, offer high translational fidelity due to preserved human genetics and rapid tumor engraftment. Studies demonstrate that EGFR inhibition in these models suppresses AKT/MAPK phosphorylation by 50–70%, reducing proliferation and inducing G1 arrest. Conversely, the DMBA-induced model in Sprague-Dawley rats mimics multistage carcinogenesis, with DMBA (a polycyclic aromatic hydrocarbon) metabolically activating to form DNA adducts, yielding heterogeneous ER-positive and ER-negative tumors after 8–12 weeks. This model captures stromal interactions and immune modulation absent in xenografts, revealing DMBA's role in deregulating EGFR/ErbB2-ER crosstalk, which accelerates chromosomal instability. Comparative analyses show DMBA tumors exhibit 40% higher baseline VEGF, reflecting angiogenic dependency, making it ideal for evaluating vascular endpoints.[4]

Despite these strengths, gaps persist in integrating dynamic biomarker monitoring with advanced analytics. Traditional immunohistochemistry (IHC) and Western blotting quantify p-AKT, p-MAPK, and VEGF but suffer from subjectivity and low throughput. Tumor regression metrics (e.g., volume, Ki-67) often overlook microenvironmental nuances like necrosis or vascular density. Enter artificial intelligence (AI)-driven histopathology, which leverages convolutional neural networks (CNNs) for automated feature extraction from whole-slide images. Tools like ImageXpert employ deep learning to segment tumor-stroma interfaces, score biomarker intensity with sub-cellular resolution, and predict outcomes with AUC >0.90. In breast cancer, AI has revolutionized risk stratification, distinguishing luminal A from basal-like subtypes via H&E alone and correlating PD-L1 expression with therapy response. A 2025 study validated AI for multiplex IHC, analyzing 11 markers in 1,400 invasive cases to forecast recurrence with 85% accuracy. Yet, applications in EGFR contexts remain underexplored, particularly for preclinical modulation studies.[5]

This study addresses these voids by translating EGFR molecular mechanisms into comprehensive in vivo validation. We hypothesize that gefitinib-mediated inhibition will elicit dose-dependent tumor regression across xenograft and DMBA models, concomitant with AKT/MAPK/VEGF suppression, quantifiable via AI-enhanced histopathology. Specific aims include: (1) Establish and characterize model-specific EGFR modulation; (2) Monitor regression dynamics and downstream biomarkers; (3) Integrate ImageXpert for unbiased histopathological phenotyping, correlating AI outputs with functional outcomes.[6]

By juxtaposing human-derived xenografts with rat autochthonous tumours, we capture spectrum-wide efficacy, from rapid TKI responses to chronic inflammatory contexts. Preliminary data suggest model discordance—xenografts showing steeper regression (60% vs. 45%)—highlighting the need for multi-model pipelines. AI integration not only amplifies reproducibility but unveils subtle changes, such as 30% increased perivascular cuffing post-inhibition, potentially prognostic for anti-angiogenic combos.[7]

Animal Models

All animal experiments were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition) and approved by the Institutional Animal Care and Use Committee (IACUC) at [Institution Name] (Protocol #2024-045). Female animals were used to align with the mammary gland biology of human breast cancer. Housing was maintained under specific pathogen-free conditions with a 12:12-h light/dark cycle, temperature of 22 ± 2°C, humidity of 50 ± 10%, and ad libitum access to standard rodent chow (Purina 5001) and autoclaved water. To minimize bias, animals were randomized using stratified block randomization based on body weight and tumor size (where applicable), and treatment allocations were blinded to investigators during endpoint assessments. Sample sizes were determined a priori via power analysis ($\alpha = 0.05$, power = 0.80, assuming 30% effect size on tumor volume) using G*Power software, yielding n = 10 per group. Humane endpoints

included body weight loss >20%, tumor ulceration, or lethargy; euthanasia was performed via CO2 inhalation followed by bilateral thoracotomy.[8]

Xenograft Model

Athymic BALB/c nude (nu/nu) female mice, aged 8–10 weeks and weighing 18–22 g, were purchased from Charles River Laboratories (Wilmington, MA, USA) and acclimated for 7 days prior to experimentation. The MDA-MB-468 human triple-negative breast cancer cell line (ATCC HTB-132; EGFR-overexpressing) was authenticated by short tandem repeat profiling (ATCC) and tested mycoplasma-free (MycoAlert, Lonza) within the prior 3 months. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich), 1% penicillin-streptomycin (Gibco), and 2 mM L-glutamine (Gibco) at 37°C in a humidified 5% CO2 atmosphere. For implantation, cells were harvested at 80–90% confluence using 0.25% trypsin-EDTA (Gibco), counted with a hemocytometer, and assessed for viability (>95%) via trypan blue exclusion. Cells were resuspended at 5 × 10^6 cells/mL in a 1:1 (v/v) mixture of phosphate-buffered saline (PBS; Gibco) and growth factor-reduced Matrigel (Corning). Each mouse received a subcutaneous injection of 200 µL (1 × 10^6 cells) into the right flank using a 1-mL syringe with a 27-gauge needle. Tumor growth was monitored twice weekly using digital calipers (Mitutoyo), with volume calculated as V = (length × width^2)/2. Mice were randomized into treatment groups when mean tumor volume reached 100–150 mm³ (typically 14–21 days post-implantation). This model recapitulates human EGFR-driven TNBC progression, with tumors exhibiting rapid growth and high vascularity suitable for efficacy studies.[9]

DMBA-Induced Model

Female Sprague-Dawley rats, aged 6–8 weeks and weighing 150–200 g, were obtained from Envigo (Indianapolis, IN, USA) and acclimated for 7 days. To induce mammary carcinogenesis, rats received four weekly oral gavage doses of 5 mg 7,12-dimethylbenz[a]anthracene (DMBA; Sigma-Aldrich, ≥95% purity) dissolved in 1 mL corn oil (Sigma-Aldrich) starting at 50 days of age, administered via a 16-gauge gavage needle under light isoflurane anesthesia (2% in oxygen). This multi-dose regimen minimizes acute toxicity while promoting heterogeneous tumor development, mimicking multistage human breast carcinogenesis. Body weights were recorded weekly, and mammary glands were palpated twice weekly from week 4 post-initiation using gloved fingers to detect palpable masses. Tumor incidence was confirmed by high-resolution ultrasonography (Vevo 3100, Fujifilm VisualSonics) if ambiguity arose. Animals were randomized upon detection of the first palpable tumor (typically 8–10 weeks post-initiation), with only those bearing at least one tumor ≥50 mm³ included. This autochthonous model yields predominantly ER-positive and ER-negative adenocarcinomas with stromal and immune components, ideal for assessing EGFR modulation in an intact microenvironment.[10]

EGFR-Targeted Agents and Treatment

Gefitinib (Iressa; AstraZeneca, Wilmington, DE, USA; ≥99% purity, lot #GD-2024-001) was selected as the EGFR tyrosine kinase inhibitor (TKI) based on its established efficacy in EGFR-overexpressing breast cancer models, with an IC50 of 33 nM against wild-type EGFR kinase activity. Stock solutions were prepared fresh weekly by dissolving gefitinib in 0.5% (v/v) methyl-2-hydroxyethyl cellulose (Sigma-Aldrich, St. Louis, MO, USA) in sterile distilled water, achieving a final concentration of 10 mg/mL, with sonication for 5 min to ensure homogeneity. Vehicle control consisted of the methylcellulose excipient alone. Dosing volume was standardized at 5 mL/kg body weight for mice and 10 mL/kg for rats to maintain consistent exposure.[11]

In the xenograft model, treatment commenced upon randomization (tumor volume 100–150 mm³) and consisted of daily oral gavage of gefitinib at 50 mg/kg for 4 weeks (28 days), administered between 8:00–10:00 AM to minimize circadian variability. This regimen aligns with pharmacokinetic data demonstrating sustained plasma levels ($C_{max} \approx$ 2–5 µM) sufficient for >80% EGFR occupancy in murine tissues, while avoiding toxicity thresholds observed at >100 mg/kg. Control mice received vehicle gavage on the identical schedule. Preliminary tolerability assessments confirmed no significant weight loss (>5%) or behavioral alterations in treated cohorts.[12]

For the DMBA-induced model, gefitinib dosing initiated upon first palpable tumor detection (volume ≥50 mm³, 8–10 weeks post-induction) at 50 mg/kg daily via oral gavage for 4 weeks, adjusted for body weight biweekly. This dose was extrapolated from rat mammary carcinogenesis prevention studies, where daily gefitinib (25–100 mg/kg) suppressed tumor multiplicity by >75% without overt hepatotoxicity. Vehicle controls followed the same protocol. Treatment compliance was verified by weighing gavage syringes pre- and post-administration, with >95% delivery confirmed across groups.[13]

Groups were as follows: (1) xenograft vehicle (n=10); (2) xenograft gefitinib (n=10); (3) DMBA vehicle (n=10); (4) DMBA gefitinib (n=10). Sample sizes were powered to detect a 30% difference in tumor volume reduction (σ=15%,

α=0.05, power=0.80) based on pilot data and historical variances. Crossover or early termination occurred if tumors exceeded 1,500 mm³ or humane endpoints were met, affecting <10% of animals. Post-treatment, mice and rats were euthanized 24 h after the final dose to capture peak pharmacodynamic effects.[14]

Tumor Monitoring and Biomarker Assays

Tumor progression and treatment response were longitudinally assessed through non-invasive and invasive endpoints to capture dynamic changes in volume, proliferation, and molecular signaling. All measurements were performed by blinded observers to mitigate bias.[15]

Tumor Volume Assessment

Tumor dimensions were measured twice weekly using digital calipers (Mitutoyo, Aurora, IL, USA; accuracy ±0.01 mm) from the initiation of treatment until study endpoint. For xenograft tumors, length (L, longest axis) and width (W, perpendicular axis) were recorded, with volume calculated using the standard ellipsoid formula: $V = (L \times W^2) / 2$. Relative tumor volume (RTV) was computed as V_t / V_0, where V_t is the volume at time t and V_0 is the baseline volume at randomization. Tumor growth inhibition (TGI) was determined as [1 - (RTV_treated / RTV_control)] × 100%. In the DMBA model, multiple tumors per animal were tracked individually if ≥50 mm³, with the largest serving as the primary endpoint; palpation was supplemented by ultrasonography (Vevo 3100, Fujifilm VisualSonics, Toronto, ON, Canada) at weeks 2 and 4 for volumetric confirmation (probe frequency 21–55 MHz, resolution <50 μm). This caliper-based approach, widely validated for xenograft efficacy studies, provides a reliable surrogate for tumor burden with low inter-observer variability (CV <10%). Animals were monitored daily for welfare, with endpoints triggered by tumor volume exceeding 1,500 mm³, ulceration, or >20% body weight loss.[16]

Necropsy and Tissue Collection

At study termination (24 h post-final dose to align with gefitinib's $T_{1/2} \approx 48$ h in rodents), animals were euthanized under deep isoflurane anesthesia (4% in oxygen) followed by exsanguination via cardiac puncture. Comprehensive necropsy was performed immediately, adhering to standardized protocols for rodent oncology models. Primary tumors were excised en bloc, with gross pathology documented photographically (Canon EOS Rebel T7, scale bar included). Tissues were divided: one portion (≈50 mg) snap-frozen in liquid nitrogen and stored at -80°C for Western blotting; adjacent sections (≈1 cm³) fixed in 10% neutral buffered formalin (NBF; Sigma-Aldrich) for 24 h at room temperature, followed by dehydration in graded ethanol, clearing in xylene, and paraffin embedding (Leica TP1020, Wetzlar, Germany) for sectioning at 4–5 μm thickness. Contralateral mammary glands, lungs, liver, and lymph nodes were similarly processed for metastasis evaluation. Fixation timing was optimized to preserve antigenicity, as prolonged exposure can attenuate phospho-epitope detection in signaling assays. All procedures minimized post-mortem autolysis, with tissues processed within 30 min of harvest.[17]

Western Blot Analysis

Frozen tumor lysates were prepared by homogenization in RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 1× Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher), followed by centrifugation at 12,000 × g for 15 min at 4°C. Protein concentration was quantified using the BCA assay (Pierce, Rockford, IL, USA) against bovine serum albumin standards, targeting 20–50 μg per lane. Samples were denatured in 4× Laemmli buffer (Bio-Rad, Hercules, CA, USA) with 5% β-mercaptoethanol at 95°C for 5 min, resolved on 10% TGX Stain-Free gels (Bio-Rad) at 120 V, and transferred to PVDF membranes (Immobilon-FL, Millipore, Burlington, MA, USA) via wet transfer (100 V, 1 h). Membranes were blocked in 5% non-fat dry milk in TBST (20 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.1% Tween-20) for 1 h, then probed overnight at 4°C with primary antibodies: rabbit anti-p-AKT (Ser473, #4060, 1:1,000; Cell Signaling Technology, Danvers, MA, USA), rabbit anti-p-MAPK (Thr202/Tyr204, #4370, 1:1,000; Cell Signaling), rabbit anti-VEGF (sc-7269, 1:500; Santa Cruz Biotechnology, Dallas, TX, USA), and mouse anti-β-actin (A5441, 1:5,000; Sigma-Aldrich) as loading control. Secondary HRP-conjugated goat anti-rabbit (111-035-003, 1:10,000; Jackson ImmunoResearch, West Grove, PA, USA) or anti-mouse (115-035-003, 1:10,000) was applied for 1 h at room temperature. Bands were visualized using Clarity Western ECL Substrate (Bio-Rad) on a ChemiDoc MP imager (Bio-Rad), with densitometry performed via Image Lab software (version 6.1; integrated optical density normalized to β-actin). This method reliably quantifies pathway activation in breast cancer models, with phospho-specific antibodies validated for rodent tissues.[18]

Immunohistochemistry and Immunofluorescence

Paraffin sections were deparaffinized in xylene and rehydrated through ethanol gradients, followed by antigen retrieval in citrate buffer (pH 6.0; 10 mM sodium citrate, 0.05% Tween-20) via heat-induced epitope retrieval (HIER) at 95°C for 20 min in a PT Link module (Dako, Santa Clara, CA, USA). Endogenous peroxidase was quenched with

3% H_2O_2 in methanol for 10 min. Slides were blocked with 5% goat serum in PBS for 1 h, then incubated overnight at 4°C with primaries: anti-p-AKT (1:200), anti-p-MAPK (1:200), anti-VEGF (1:100), and rabbit anti-Ki-67 (ab15580, 1:500; Abcam, Cambridge, UK) for proliferation. For immunofluorescence (IF), Alexa Fluor 488-conjugated goat anti-rabbit secondary (A-11008, 1:1,000; Thermo Fisher) was used, with DAPI counterstain (1 μg/mL; Sigma-Aldrich) for nuclei. IHC employed biotinylated secondary (Vector Laboratories, Burlingame, CA, USA) amplified via ABC Elite kit, developed with DAB chromogen (Dako), and counterstained with hematoxylin. Slides were dehydrated, mounted in Permount (Fisher Scientific, Hampton, NH, USA), and imaged at 20×/40× on an Aperio AT2 scanner (Leica Biosystems, Wetzlar, Germany).[19]

AI-Based Histopathology Analysis

Digital histopathological analysis was performed using ImageXpert AI software (version 5.2.1; ImageXpert Inc., Nashua, NH, USA), a deep learning-enabled platform designed for quantitative tissue phenotyping in oncology research. ImageXpert integrates convolutional neural networks (CNNs) and U-Net architectures for automated feature extraction from whole-slide images (WSIs), offering scalability for high-throughput biomarker assessment. The software was installed on a dedicated workstation (Dell Precision 7820; Intel Xeon Gold 6248R CPU, 128 GB RAM, NVIDIA RTX A6000 GPU with 48 GB VRAM) running Ubuntu 22.04 LTS, with CUDA 12.1 for GPU acceleration. This setup processes 20× WSIs (≈1 GB each) in <5 min per slide, enabling batch analysis of up to 100 slides overnight.[20]

Slide Digitization and Preprocessing

Formalin-fixed, paraffin-embedded (FFPE) tumor sections (4–5 μm) were stained with hematoxylin and eosin (H&E) or subjected to IHC/IF as described previously. Slides were scanned at 40× magnification (0.25 μm/pixel resolution) using an Aperio GT 450 S2 digital scanner (Leica Biosystems, Wetzlar, Germany), generating uncompressed TIFF files in SVS format. Preprocessing in ImageXpert included automated tile extraction (512×512 pixel patches), color normalization via Macenko's method to mitigate staining variability (e.g., hematoxylin OD normalization to a reference slide from a treatment-naïve xenograft), and artifact removal (e.g., tissue folds, bubbles) using a pre-trained ResNet-50 classifier (threshold >0.95 confidence). This step ensured input consistency, as staining batch effects can confound AI predictions by up to 20% in multi-site studies.[21]

AI Algorithms for Feature Quantification

ImageXpert employed a multi-task CNN framework for simultaneous segmentation and classification. Tumor cell segmentation utilized a modified U-Net with attention gates, trained to delineate epithelial compartments, stroma, and necrosis based on morphological features (e.g., nuclear density, texture entropy). Necrosis scoring was derived from a binary classifier (necrotic vs. viable tissue) integrated with spatial aggregation, yielding a necrosis index (NI) as the percentage of necrotic area within the tumor mask (range 0–100%). For biomarker analysis, IHC/IF slides were processed via a multiplexed object detection model (YOLOv8 backbone fine-tuned on DAB/Alexa Fluor signals), quantifying intensity as mean optical density (MOD) for p-AKT, p-MAPK, and VEGF, and proliferation via Ki-67-positive nuclear counts per mm². Vascular density was assessed by thresholding CD31 co-stains (if applicable) to compute microvessel area fraction. All models incorporated ensemble averaging (5-fold) to reduce overfitting, with hyperparameter tuning via grid search on validation sets (learning rate 1e-4, Adam optimizer, batch size 16).[22]

Model Training and Validation

Algorithms were trained on a curated dataset of 500 annotated WSIs (250 xenograft-derived, 250 DMBA-induced; 70/15/15 train/validation/test split) from independent cohorts, annotated by two board-certified pathologists using QuPath v0.4.4 (University of Edinburgh) for ground truth (inter-annotator κ=0.87). Annotations included pixel-level masks for segmentation (n=200 slides) and region-level scores for biomarkers (n=300 slides). Training incorporated data augmentation (rotations, flips, elastic deformations) and class balancing for rare events like necrosis (prevalence <15%). Performance metrics included Dice similarity coefficient (DSC) for segmentation (mean 0.92 for tumor, 0.88 for necrosis), area under the receiver operating characteristic curve (AUC) for classification tasks (AUC=0.91 for Ki-67 positivity, 0.89 for VEGF intensity), and mean absolute error (MAE<5%) for continuous scores. Validation against an external test set (100 WSIs from a public TCGA-BRCA cohort) confirmed generalizability (DSC drop <3%, p=0.12 by paired t-test). These benchmarks exceed thresholds recommended for clinical-grade AI tools, ensuring reliability in preclinical contexts.[23]

Workflow Integration and Manual Correlation

The end-to-end workflow commenced with batch upload of SVS files to ImageXpert's cloud-synced interface, followed by automated preprocessing (≈10 min/batch), AI inference (parallelized across GPU cores), and export of

quantitative reports in CSV/JSON formats. Outputs were overlaid on WSIs for visual inspection (e.g., heatmaps for biomarker heterogeneity). To validate AI against manual scoring, a subset of 40 slides (10/group) was independently quantified by pathologists using a semi-quantitative H-score (0–300 scale for intensity × % positivity). Concordance was assessed via Pearson's correlation coefficient ($r=0.92$ for necrosis index, $r=0.89$ for Ki-67; both $p<0.001$) and Bland-Altman plots, revealing minimal bias (<2% mean difference). Discrepancies were adjudicated via consensus review, with AI outperforming manual in reproducibility (CV 4% vs. 12%). This integration facilitates seamless correlation of AI-derived metrics with functional endpoints, such as linking NI to tumor regression (Spearman's $\rho=0.76$). All analyses were conducted blinded to treatment groups, with code availability upon request via GitHub repository[24]

Establishment and Characterization of Preclinical Models

Both preclinical models were successfully established with high reproducibility, recapitulating key features of EGFR-driven breast cancer progression. In the xenograft model, MDA-MB-468 cells engrafted in 100% of athymic nude mice (n=20), with palpable tumors detectable by day 7 post-implantation. Tumor growth followed a sigmoidal pattern, reaching randomization criteria (100–150 mm^3) by day 14 ± 2. Mean baseline tumor volume was 128 ± 18 mm^3, with no significant differences between future treatment groups ($p=0.87$, one-way ANOVA). Over the pre-treatment period, tumors expanded at a rate of 25 ± 5% per week, consistent with the aggressive phenotype of EGFR-overexpressing TNBC lines.[25]

The DMBA-induced model yielded tumors in 85% of Sprague-Dawley rats (n=20), with the first palpable masses emerging at 8.2 ± 1.1 weeks post-initiation. By randomization (week 9–10), 14/20 rats developed multifocal mammary adenocarcinomas, with the primary tumor averaging 72 ± 12 mm^3. Growth kinetics were more variable than xenografts, reflecting the heterogeneous, multistage carcinogenesis, with a mean weekly increase of 18 ± 6% and occasional spontaneous regressions (<10% incidence). Ultrasonography confirmed tumor vascularity, with Doppler signals indicating peak systolic velocity of 15–20 cm/s in viable regions.[26]

Comparative growth trajectories are depicted in Figure 1A, illustrating the steeper exponential phase in xenografts (doubling time 5.2 days) versus the linear progression in DMBA tumors (doubling time 7.8 days; log-rank $p<0.01$ for growth curves). Histological examination at baseline revealed ductal adenocarcinomas in both models, with xenografts exhibiting higher mitotic indices (Ki-67 positivity 45 ± 8%) compared to DMBA (32 ± 7%; $p=0.02$, unpaired t-test), underscoring the immunodeficient versus immunocompetent contexts.[27]

Baseline EGFR characterization confirmed overexpression as a unifying feature. Western blot analysis of pre-treatment lysates showed EGFR protein levels 4.2-fold higher in xenografts relative to normal mammary epithelium (densitometry normalised to β-actin; Figure 1B), aligning with the cell line's genomic amplification (EGFR copy number >10 by FISH). In DMBA tumors, EGFR expression was elevated 2.8-fold over controls, with IHC revealing membranous staining in 65 ± 9% of neoplastic cells (H-score 180 ± 22), predominantly in ER-negative foci (Figure 1C). Phospho-EGFR (Y1068) was detectable in 55% of xenograft samples versus 40% in DMBA ($p=0.04$), correlating with baseline p-AKT levels ($r=0.72$, Pearson). These profiles validate the models' suitability for EGFR modulation studies, with xenografts modelling rapid, human-derived responses and DMBA capturing chronic, inflammation-associated deregulation.[28]

EGFR Modulation and Tumor Regression

Gefitinib treatment elicited potent EGFR inhibition across both models, as evidenced by rapid pharmacodynamic changes in receptor phosphorylation. In xenografts, Western blot analysis of tumors harvested 24 h post-first dose revealed a 78 ± 11% reduction in phospho-EGFR (Y1068) levels relative to vehicle controls (densitometry normalized to total EGFR and β-actin; $p<0.001$, unpaired t-test; Figure 2A). This suppression persisted through the 4-week regimen, with IHC confirming diminished membranous staining (H-score 45 ± 8 in treated vs. 185 ± 21 in controls; $p<0.001$). Similarly, in DMBA tumors, p-EGFR decreased by 61 ± 14% ($p<0.01$), though with greater inter-animal variability (CV 23% vs. 14% in xenografts), potentially attributable to stromal heterogeneity.[29]

Tumor regression was dose- and model-dependent, with gefitinib inducing marked growth stasis followed by cytoreduction. In the xenograft model, treated tumors exhibited an initial 15% volume contraction by week 1, culminating in a net 62% reduction from baseline by week 4 (final mean volume 49 ± 12 mm^3 vs. 327 ± 45 mm^3 in controls; $p<0.001$, two-way repeated-measures ANOVA; Figure 2B). Tumor growth inhibition reached 89% at endpoint, with 40% of treated animals achieving complete responses (undetectable tumors <20 mm^3). Kaplan-Meier analysis of progression-free survival (defined as volume <200% baseline) showed median survival of 28 days in treated vs. 14 days in controls (hazard ratio 0.22, 95% CI 0.12–0.41; log-rank $p<0.0001$;).[30]

The DMBA model displayed attenuated but significant efficacy, with a 48% volume reduction (final mean 38 ± 9 mm^3 vs. 73 ± 15 mm^3; $p<0.01$). Regression onset was delayed (week 2), reflecting slower proliferation kinetics, and TGI was 72% (Figure 2B). No complete responses occurred, but multifocal tumors regressed uniformly (mean reduction across lesions 51 ± 10%). Comparative metrics are summarized in Table 1, highlighting xenografts' superior sensitivity (effect size Cohen's d=2.1 vs. 1.4 in DMBA). Dose-response curves, derived from a parallel cohort receiving 25 or 100 mg/kg, demonstrated an EC50 of 42 mg/kg for volume inhibition in xenografts, plateauing at 50 mg/kg (Figure 2D). These data affirm gefitinib's capacity to modulate EGFR and drive regression, with model-specific nuances informing translational strategies.[31]

Downstream Pathway Inhibition

Gefitinib-mediated EGFR blockade translated into profound suppression of canonical downstream effectors, corroborating the mechanistic link from receptor inhibition to cytostatic and cytotoxic outcomes. Western blot quantification of endpoint tumor lysates demonstrated a 68 ± 9% reduction in phosphorylated AKT (Ser473) in gefitinib-treated xenografts compared to vehicle ($p<0.001$, unpaired t-test; normalized to total AKT; Figure 3A). This attenuation was mirrored in the DMBA model (55 ± 12% decrease; $p<0.01$), with a trend toward greater residual activation in autochthonous tumors ($p=0.06$ for model interaction, two-way ANOVA). Similarly, p-MAPK (Thr202/Tyr204) levels declined by 52 ± 10% in xenografts ($p<0.001$) and 44 ± 11% in DMBA ($p<0.05$; Figure 3B), consistent with ERK-mediated feedback loops in EGFR signaling.[32]

VEGF expression, a key angiogenic mediator, exhibited dose-sensitive downregulation, with 42 ± 8% lower levels in treated xenografts ($p<0.01$) versus 31 ± 9% in DMBA ($p<0.05$; Figure 3C). Time-course analysis from supplementary cohorts (Supplementary Figure S1) revealed peak inhibition at week 2 (e.g., 75% p-AKT reduction), followed by partial rebound by week 4 (to 45%), suggestive of adaptive resistance mechanisms. IHC corroborated these findings spatially: in xenografts, p-AKT staining intensity (mean fluorescence intensity, MFI) dropped 61% in neoplastic cells (from 145 ± 18 to 57 ± 12 arbitrary units; $p<0.001$; Figure 3D, left panel), with comparable shifts for p-MAPK (48% reduction) and VEGF (39%; nuclear translocation attenuated). DMBA tumors showed patchy suppression, with 52% fewer p-AKT-positive cells in periductal regions ($p<0.01$; Figure 3D, right panel), highlighting microenvironmental influences.[33]

Correlative analyses underscored pathway interdependence: changes in p-EGFR correlated strongly with p-AKT ($r=0.81$, Pearson) and VEGF ($r=0.69$) across models, while p-MAPK reductions associated with Ki-67 declines ($r=0.76$). These data establish gefitinib's efficacy in decoupling EGFR-driven survival and proliferative signals, with quantitative metrics supporting biomarker utility for response prediction.[34]

AI-Enhanced Histopathological Insights

Integration of ImageXpert AI unveiled nuanced histopathological alterations beyond manual capabilities, quantifying treatment-induced remodeling with high fidelity. In H&E-stained WSIs, AI-derived necrosis index (NI) surged 35 ± 7% in gefitinib-treated xenografts (from 12 ± 3% to 47 ± 5%; $p<0.001$) and 28 ± 6% in DMBA (to 36 ± 4%; $p<0.01$; Figure 4A), manifesting as coalescent acellular zones with pyknotic debris. Tumor-stroma segmentation revealed a 22% contraction in epithelial compartment area ($p<0.05$), concomitant with stromal expansion, indicative of desmoplastic remodeling.[35]

For biomarker phenotyping, AI quantified Ki-67-positive nuclei at 1,250 ± 180/mm^2 in control xenografts, plummeting to 620 ± 95/mm^2 post-treatment (50% reduction; $p<0.001$; Figure 4B). Concordance with manual H-scores was robust ($r=0.88$, $p<0.001$), yet AI detected spatial heterogeneity—e.g., 40% lower Ki-67 in hypoxic cores (oxygen tension proxy via entropy mapping)—unapparent in pathologist reads. p-AKT intensity (MOD) fell 59% ($p<0.001$), aligning with Western data, while VEGF MFI decreased 38%, correlating with reduced microvessel density (19% drop in CD31 co-stains; Spearman's $\rho=0.82$;).[36]

Representative overlays (Figure 4D) illustrate AI's precision: color-coded heatmaps highlight necrotic expansion (red) and biomarker quenching (blue gradients) in treated sections. Table 2 enumerates model-stratified metrics, showing xenografts' heightened sensitivity (e.g., NI effect size d=3.2 vs. 2.1 in DMBA). Bland-Altman analysis affirmed minimal bias (mean difference -1.2% for NI; limits of agreement ±8%), with AI accelerating throughput (100 slides analyzed in 4 h vs. 20 h manual). These insights not only validate pathway inhibition histologically but reveal AI's role in discerning subtype-specific responses, such as DMBA's persistent perivascular VEGF, informing combinatorial strategies.[37]

Discussion

This study provides compelling in vivo evidence for the translational potential of EGFR-targeted therapy in breast cancer, bridging molecular mechanisms to measurable efficacy endpoints through complementary preclinical models. Gefitinib, at a clinically relevant dose of 50 mg/kg, achieved robust EGFR modulation, yielding 62% tumor volume reduction in MDA-MB-468 xenografts and 48% in DMBA-induced rat tumors. These regressions were underpinned by significant suppression of downstream AKT (68% in xenografts), MAPK (52%), and VEGF (42%) signaling, as quantified by Western blot and IHC. The integration of ImageXpert AI further illuminated histopathological dynamics, revealing a 35% increase in necrosis index and 50% drop in Ki-67 proliferation, with high concordance to manual scoring (r=0.91). Model-specific disparities—steeper responses in xenografts versus delayed but sustained effects in DMBA—highlight the value of multi-model validation, capturing both human-like rapid progression and autochthonous inflammatory contexts.[38]

These findings align with and extend prior preclinical data on EGFR inhibitors in breast cancer. For instance, early studies in basal-like models demonstrated EGFR TKIs' capacity to halt proliferation via AKT/MAPK blockade, yet often overlooked angiogenic endpoints like VEGF, which our work quantifies as a critical mediator of incomplete responses. In DMBA models, chemical induction mimics estrogen-independent tumorigenesis, where EGFR/ErbB crosstalk amplifies resistance; our observed 61% p-EGFR reduction nonetheless curbed this, suggesting therapeutic windows even in heterogeneous lesions. The xenograft's superior sensitivity (TGI 89% vs. 72%) recapitulates clinical heterogeneity, where TNBC subsets respond better to TKIs than luminal types, informing patient stratification strategies.[39]

Mechanistically, gefitinib's efficacy stems from disrupting EGFR's pleiotropic signaling, as evidenced by strong correlations between p-EGFR and downstream markers (r=0.81 for p-AKT). Inhibition induced G1 arrest and apoptosis, inferred from Ki-67 declines and necrosis expansion, while VEGF downregulation mitigated angiogenesis—evident in 19% reduced microvessel density. Time-course data (Supplementary Figure S1) unmasked adaptive rebound by week 4, with partial p-AKT recovery, echoing resistance via PI3K feedback loops reported in EGFR-high breast cancers. Model differences further elucidate context: xenografts, lacking immune stroma, showed uniform suppression, whereas DMBA tumors exhibited patchy VEGF persistence in perivascular niches, potentially driven by macrophage-derived factors. This underscores EGFR's role not only in epithelial signaling but in tumor-stroma crosstalk, a nuance traditional assays undervalue.[40]

A pivotal innovation herein is the seamless incorporation of AI-based histopathology via ImageXpert, which transcended manual limitations in precision and scalability. AI segmentation achieved Dice scores >0.90 for tumor/necrosis boundaries, enabling sub-cellular quantification of biomarker heterogeneity—e.g., 40% lower p-AKT in hypoxic cores—that manual H-scoring missed (CV 4% vs. 12%). Recent advances in AI for breast pathology corroborate this, with deep learning models enhancing subtype classification and recurrence prediction in WSIs. By processing 100 slides in hours, ImageXpert facilitates high-throughput preclinical screening, reducing observer bias and accelerating endpoint correlation (e.g., NI ρ=0.76 with regression). Limitations include algorithm training on annotated datasets, risking overfitting to specific stains; however, external TCGA validation (DSC drop <3%) bolsters generalizability. This AI augmentation not only validates pathway inhibition but unveils prognostic features, like stromal remodeling, positioning it as a cornerstone for next-generation oncology pipelines.[41]

Clinically, these insights hold promise for revitalizing EGFR TKIs in breast cancer, where phase II trials have yielded modest 8–20% response rates due to poor stratification. Our biomarkers—p-AKT/VEGF reductions >50%—could serve as pharmacodynamic surrogates in trials like the ongoing EGFR-enriched TNBC cohorts (NCT04539938), potentially identifying responders via liquid biopsies. The DMBA model's immunocompetent milieu suggests applicability to immunotherapy combos, as EGFR inhibition may enhance T-cell infiltration by normalizing vasculature. A 2025 meta-analysis emphasizes such multi-omics integration for overcoming resistance, aligning with our AI-driven phenotyping. Ultimately, these data advocate for biomarker-led trials, narrowing the preclinical-clinical gap and accelerating precision therapies.[42]

Notwithstanding these advances, limitations warrant acknowledgment. Preclinical models, while robust, incompletely mirror human disease: xenografts lack systemic immunity, potentially inflating efficacy, while DMBA's rat physiology diverges in metabolism (gefitinib clearance 20% faster). Sample sizes (n=10/group), though powered for primary endpoints, limit subgroup analyses (e.g., ER+ vs. ER- DMBA foci). Gefitinib monotherapy sidesteps combination synergies, as clinical resistance often involves MET/AXL bypasses; future studies must address this. AI's reliance on high-quality WSIs assumes standardized pathology, which varies across labs. Finally, translatability hinges on human EGFR pharmacodynamics, where polymorphisms alter sensitivity.[43]

Looking ahead, these findings propel multifaceted directions. Combining gefitinib with anti-VEGF agents (e.g., bevacizumab) could synergize anti-angiogenic effects, testable in advanced PDX models. Longitudinal AI tracking—e.g., serial WSIs for resistance evolution—promises real-time adaptive dosing. Expanding to

CRISPR-edited models with EGFR mutants could probe rare variants, while federated AI learning across consortia enhances algorithm robustness. By fusing mechanism, efficacy, and analytics, this framework equips the field to conquer EGFR's therapeutic enigmas in breast cancer.[44]

Conclusion

In summary, this investigation delineates a mechanistic continuum from EGFR inhibition to tangible in vivo efficacy in breast cancer models, affirming gefitinib's prowess in eliciting tumor regression through AKT/MAPK/VEGF suppression. The dual-model approach—xenografts for translational fidelity and DMBA for ecological validity—illuminates efficacy spectra, with quantitative histopathology via ImageXpert AI providing unprecedented resolution into necrosis, proliferation, and remodeling dynamics. These results not only validate EGFR as a druggable node in TNBC and beyond but underscore AI's transformative role in preclinical rigor, mitigating subjectivity and unveiling subtle biomarkers for response forecasting.

By translating molecular insights into actionable endpoints, our work bridges a critical chasm in oncology drug development, where historical discrepancies have stymied TKI advancement. The observed 62% xenograft regression and correlated pathway quiescence herald potential for biomarker-stratified regimens, particularly in EGFR-high subsets resistant to standard care. AI integration exemplifies a paradigm shift, enabling scalable, reproducible phenotyping that could standardize trial readouts and personalize therapies.

We urge the adoption of AI-augmented pipelines in preclinical oncology, fostering collaborations between computational biologists and pharmacologists. As breast cancer mortality persists—projected at 700,000 global deaths by 2030—such innovations are imperative to harness EGFR's untapped potential, ultimately delivering precision cures to patients.

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