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EGFR Expression in Breast Cancer Association with biologic phenotype and clinical outcomes

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Abstract

Background—EGFR expression is associated with aggressive phenotypes in preclinical breast cancer models, but in clinical studies, EGFR has been inconsistently linked to poor outcome. We hypothesized that EGFR expression in human breast tumors, when centrally and uniformly assessed, is associated with an aggressive phenotype and resistance to systemic therapy.

Methods—In a database of 47,286 patients with breast cancer, EGFR status was known on 2,567. EGFR levels were measured centrally by ligand binding assay, and tumors with ≥10 fmol/mg were prospectively deemed positive. Clinical and biological features of EGFR positive and negative tumors were compared. Clinical outcomes were assessed by systemic therapy status.

Results—475 out of 2,567 tumors (18%) were EGFR positive. EGFR-positive tumors were more common in younger and in black women, were larger, had a higher S-phase fraction, and were more likely to be aneuploid. EGFR positive tumors were more likely to be HER2-positive (26% vs. 16%, p<0.0001), but less likely to be ER-positive (60% vs. 88%, p<0.0001), or PR-positive (26% vs. 65%, p<0.0001).

In multivariate analyses, EGFR expression independently correlated with worse DFS (HR=1.66, 95% CI=1.4–2.41, p=0.007) and OS (HR=1.98, 95% CI=1.36–2.88, p=0.0004) in treated patients, but not in untreated patients.

Conclusions—EGFR expression is more common in breast tumors in younger and black women. It is associated with lower hormone receptor levels, higher proliferation, genomic instability, and HER2 over-expression. It is correlated with higher risk of relapse in patients receiving adjuvant treatment. Blocking EGFR may improve outcome in selected patients.

Keywords

Breast cancer; EGFR; resistance to endocrine therapy; chemotherapy resistance

Introduction

The epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) is a tyrosine kinase receptor of the epidermal growth factor receptor family. Other members are HER2, HER3, and HER4. These receptors are activated by ligand-dependent homo-and heterodimerization, which lead to kinase activation and initiation of signaling. Interaction

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between these four receptors and their various ligands may regulate and modulate their effects on cell growth and survival [1].

EGFR expression, biological impact, and influence on clinical outcome have been reported in several tumor types [2,3]. In preclinical models of breast cancer, overexpression of EGFR leads to malignant transformation of mouse cells. It is associated with increased proliferation and resistance to apoptosis [4]. Additionally, its role in resistance to hormone therapy through crosstalk with estrogen receptor (ER) is now better understood [5].

There have also been multiple reports on EGFR in clinical breast cancer. The studies were mostly small in size, used variable methodologies, and had inconsistent results [6–15]. This may be explained in part by the many challenges facing studies of EGFR in clinical breast cancer samples. There has been little standardization of analytic techniques, and most studies used formalin fixation of tissue, a process which can vary considerably between institutions and may alter antigen detectability. Moreover, immunohistochemical staining of EGFR has had inconsistent results, and is not a functional assay. Radiolabeled ligand-binding is a functional assay but is time-consuming and requires frozen tissue, which is not widely collected or banked anymore in the United States. Antigen detection is less likely to be altered in frozen tissue.

We hypothesized that EGFR expression, when centrally and uniformly measured in frozen tissue obtained from a large number of patients, would be associated with an aggressive phenotype of breast cancer and would confer resistance to systemic adjuvant therapy. We tested these hypotheses using a large frozen tissue breast tumor bank in which EGFR was assessed by a central laboratory using ligand-binding assay, and results were correlated with clinical and biologic tumor characteristics as well as clinical outcomes.

Methods

Patient Data and Specimens

The Lester and Sue Smith Breast Center at Baylor College of Medicine maintains a database of breast cancer patients whose tissue specimens were originally sent to a central reference laboratory for steroid receptor assays at the Nichols Institute in California serving more than 370 institutions throughout the United States. Demographics were reported by the patients and staff at each contributing institution. Follow-up information as well as clinical and pathologic characteristics were obtained from tumor registries, medical records, or by data collection forms completed by the office staff of the referring physicians. This database contains information on 47,286 patients diagnosed between 1984 and 1999 with early breast cancer (stage I–IIIA). This repository has been reviewed by Institutional Review Boards at the University of Texas Health Science Center at San Antonio and at Baylor College of Medicine and both boards provided a waiver of informed consent. No patient identifiers were provided to the authors.

Prognostic Factors

EGFR levels were measured by radioligand binding assay. Tumor specimens were pulverized in liquid nitrogen, homogenized, and centrifuged. Cytosol fractions and suspended fat were removed. Membrane fraction samples were incubated with fixed concentrations of radiolabeled EGF and varying concentrations of unlabeled EGF. Total protein was determined by the Lowry method [16]. Levels of ≥10 fmol/mg of membrane protein were prospectively deemed positive as in previous studies [17].

HER2 status was determined by Western blotting, using a rabbit polyclonal antibody directed against the C-terminus of the HER2 protein [18]. The cutoff value for HER2 expression was set at 1U/μg protein based on prior studies [18,19].

ER levels were measured by the dextran-coated charcoal method, as previously described [20]. From 1970 to 1984, PR levels were measured by sucrose density gradient [21]. In 1985, the standard multipoint dextran-coated charcoal assay incorporated [^{125}I]-estradiol and [^{3}H]-R5020 in a single assay, allowing the simultaneous determination of ER and PR. Samples containing at ≥3 fmol/mg protein were prospectively considered ER-positive, and those containing ≥5 fmol/mg protein were considered PR-positive, based on prior clinical studies [22–24].

DNA ploidy and S-phase fraction were evaluated by flow cytometry and the histograms were analyzed by Modfit (Verify Software House, Topsham, ME) using single-cut, debris stripping [25]. Cutoff points were determined by calibrating S-phase fraction with clinical outcome in a group of more than 28,800 patients with breast cancer (low, <6%; intermediate, 6–10%; high >10%) [26].

Statistical Methods

Patient and tumor characteristics were summarized in the 2,567 patients with EGFR data, and the relationships between these characteristics and EGFR status were examined using descriptive statistics and the chi-squared test. Then, the study dataset was compared to the 44,719 patients without EGFR data in the overall dataset using descriptive statistics, the chi-squared test, and Fisher's exact test.

Disease-free survival (DFS) was calculated from the diagnostic biopsy date and a first recurrence was scored as an event while patients without an event were censored at the time of death or last follow-up. Overall survival (OS) was calculated from the diagnostic biopsy date and death was scored as an event whereas patients who were alive at the time of last follow-up were censored. Post-relapse survival was calculated from the recurrence date in patients who had a relapse, and death was scored as an event while patients who were alive at the time of last follow-up were censored.

The effects of EGFR status on disease-free, post-relapse, and overall survival were examined by treatment group using Kaplan-Meier curves, and differences in survival were evaluated with the log-rank test. Univariate Cox regression was used to model the effect of EGFR on survival in treatment subgroups. Multivariate analysis assessed the simultaneous importance of EGFR status and other patients and tumor characteristics on DFS and OS by treatment group and Cox regression was used to model the relationships. Final multivariate models were fitted using backwards selection and adjusted hazards ratios, 95% confidence intervals, and p-values were calculated for the final models. All analyses were performed using SAS 9.1 (SAS, Cary, NC) and Stata (Statacorp, College Station, TX).

Results

From a total of 47,286 patients with breast cancer in this database, EGFR status was known on 2,567 tumors. We compared this subset to the entire database and found that it was representative of the patient population (Table 2). Tumors without EGFR data were less likely to over-express HER2 though this was probably due to the play of chance in a small sample size (n=56). Eighteen percent of the dataset we studied over-expressed HER2, which is the expected rate in breast cancer.

Of the tumors with known EGFR status, 18% (n=475) were EGFR-positive (≥10 fmol/mg membrane protein) and 82% (n=2092) were EGFR-negative (≤10 fmol/mg). Levels varied widely among EGFR-positive tumors (10–11084 fmol/mg), but 90% of them had concentrations less than 100 fmol/mg (Figure 1).

Clinical and Biologic Characteristics

Table 1 compares demographic and clinical characteristics between EGFR-positive and negative tumors. Notably, EGFR-positive tumors were more common in women younger than 50 years (40% vs. 24%). Additionally, there were more black women with EGFR-positive than EGFR-negative tumors. EGFR signaling may be a contributing reason to the worse prognosis in these groups.

EGFR-positive tumors were also more likely to be associated with adverse pathologic or biologic characteristics compared to EGFR-negative tumors (Table 1). They were more likely to be larger than 2cm, (64% vs. 46%), aneuploid (68% vs. 46%), and modestly more likely to be node-positive. Strikingly, EGFR-positive tumors were more than twice as likely to have a high proliferative fraction as EGFR-negative tumors, 53% vs. 22% respectively.

HER2 and Hormone Receptor Expression

Other biomarkers were studied to investigate how EGFR correlates to other prognostic markers. EGFR expressing tumors were more likely to over-express HER2 than EGFR-negative tumors, (26% vs. 16%), further indicating the more aggressive nature of this phenotype.

Preclinical and clinical evidence has indicated that growth factor receptor signaling can downregulate hormone receptor expression [6–9,11–15,27–34]. We therefore examined the relationship of ER and PR status to EGFR expression in this dataset. EGFR-positive tumors were less likely to express ER than EGFR-negative tumors (60% vs. 88%), and only 26% of EGFR-positive tumors expressed PR compared to 65% of EGFR-negative tumors. EGFR-positive tumors were also more likely to be hormone receptor negative than EGFR-negative tumors (37% vs. 9%).

Currently, there is substantial interest in the ER-negative, PR-negative, and HER2 negative subset of breast cancer. EGFR-positive tumors were four times more likely to have this “triple-negative” phenotype than EGFR-negative tumors (29% vs. 7%). Of 284 patients in our dataset with triple-negative tumors, 48% expressed EGFR (data not shown).

Clinical Outcomes

In our data set, 1068 patients (46%) did not receive any systemic adjuvant therapy and 1256 (54%) did: 529 (23%) received chemotherapy, 497 (21%) hormonal therapy, and 230 (10%) received both. Of patients receiving hormonal therapy, 97% got tamoxifen.

To separate any prognostic effect of EGFR from prediction of treatment effect, outcome was analyzed separately for systemically treated vs. untreated patients. In patients who were not treated with systemic adjuvant therapy, DFS was not significantly different between EGFR-positive and negative groups (HR=1.37, 95%CI=0.85–2.21, p=0.20) (Figure 2A). In contrast, patients who received adjuvant therapy had a significantly worse DFS in the EGFR expressing group (HR=1.77, 95%CI=1.36–2.29, p<0.0001) (Figure 2B), suggesting that EGFR expression may be associated with resistance to some forms of systemic therapy. Findings were very similar in OS analysis as well (data not shown).

We then examined DFS by different treatment types. In patients who received only chemotherapy, we found only a non-significant trend towards worse DFS in the EGFR expressing group (HR=1.30, 95%CI=0.91–1.86, p=0.15) (Fig 3A). In patients who received only hormonal therapy, the EGFR-positive group had a significantly worse disease free survival (HR=1.99, 95%CI=1.13–3.49, p=0.015) (Fig 3B). In patients who received both hormonal therapy and chemotherapy, the difference in DFS by EGFR status was even more prominent (HR=2.20, 95%CI=1.30–3.71, p=0.003) (Fig 3C).

Similar patterns were also found in OS in these groups. Specifically, patients who were EGFR-positive and received only chemotherapy had worse OS (HR=1.56, 95%CI=1.09–2.23, p=0.0142), and those who received both hormone therapy and chemotherapy had worse OS than EGFR-negative patients (HR=2.97, 95%CI=1.77–4.99, p<0.0001).

In the subgroup of patients who experienced relapse, patients with EGFR-positive tumors had significantly worse post-relapse survival than patients with EGFR-negative tumors regardless of their treatment status prior to relapse (17 months vs. 28 months, respectively, HR=1.72, 95%CI=1.34–2.20, p<0.0001) (Fig 4).

In multivariate analyses by treatment group, the role of EGFR as a prognostic variable for disease-free survival DFS and OS was evaluated. Variables included were: age, race, tumor size, nodal status, ER, PR, HER2, S-phase fraction, DNA ploidy, and EGFR status. In untreated patients, EGFR was not associated with DFS or OS after adjusting for the effect of other characteristics *(data not shown)*. However, in patients treated with systemic adjuvant therapy, EGFR expression was independently associated with worse DFS and OS. EGFR-positive patients had 66% higher risk of relapse and almost double the risk of death than EGFR-negative patients (DFS adjusted HR=1.66, 95%CI=1.15–2.41, p=0.0074; OS adjusted HR=1.98, 95%CI=1.36–2.88, p=0.0004).

Discussion

This is the largest and most comprehensive study to date analyzing EGFR expression in breast cancer patients. It demonstrates that EGFR expression is associated with an aggressive phenotype with distinct clinical and biologic characteristics. EGFR expressing tumors occurred more often in young and minority women. These tumors were larger and slightly more likely to metastasize to the lymph nodes. They had a substantially higher proliferation rate and greater genomic instability. They were also more likely to co-express HER2 but much less likely to express hormone receptors, especially PR.

EGFR has been extensively studied in the preclinical and clinical settings in different types of cancer. In breast cancer, preclinical evidence has indicated its clear role in increasing proliferation, resistance to apoptosis, and ligand-independent activation of the estrogen receptor, and in mediating hormone therapy resistance [4,35,36]. Additionally, activating mutations of pathway elements downstream of EGFR in cancer cells may render them resistant to EGFR inhibition [37]. This was recently shown in colon and lung cancers [38–40].

In the clinical setting, multiple studies have examined EGFR expression in breast cancer, but results have varied possibly due to relatively small sample sizes and non-uniform methodologies for the measurement of EGFR [31,37,41]. These studies reported that EGFR is expressed in 15%– 45% of breast tumors, is inversely related to hormone receptor expression, and they inconsistently linked EGFR expression to poor prognosis [6–15,41,42]. A recent study utilized a quantitative immunofluorescence-based technology to measure EGFR in a subset of premenopausal women treated with adjuvant tamoxifen [43]. The authors found EGFR in 39% of the tumors they tested, and linked its expression to higher

tumor grade and worse clinical outcome in patients treated with tamoxifen but not in untreated patients. However, that study found no correlation with tumor size, lymph node status or co-expression of HER2. This technology is promising and warrants further study.

The present study sought to overcome these deficiencies by analyzing frozen tumors from a large number of patients. EGFR expression was objectively quantified by ligand binding assay and all measurements were performed at a central lab with strict quality control.

Only a minority of breast cancer specimens (18%) expressed EGFR and apparently only low levels are needed to exert an effect on biologic and clinical characteristics. The association with a different biology, such as down-regulation of ER or PR, was strong– 37% of EGFR-positive tumors were negative for both ER and PR compared to only 9% of EGFR-negative tumors, representing a fourfold difference. b

This study provides further evidence of the inverse relation between hormone receptor expression and growth factor receptor expression and signaling, in this case EGFR. This relationship was most pronounced with PR expression, where only a quarter of EGFR-positive tumors expressed PR compared to almost two thirds of the EGFR-negative tumors.

In recent years, DNA microarray profiling studies on breast tumors defined distinct subtypes of breast carcinomas and linked them to varying clinical outcomes [44,45]. Basal-type breast cancer is one such subtype that has been associated with EGFR expression [46,47] and a poorer prognosis [44,48]. The triple-negative phenotype (ER-negative, PR-negative, HER2-negative) is considered a surrogate for this basal type breast cancer. In our study, nearly half (48%) of these triple-negative tumors were EGFR-positive compared to 18% of the overall study tumors. These findings are also consistent with other studies of triple-negative or basal tumors which also found a higher incidence of EGFR expression [46,47].

EGFR expression was positively correlated with HER2 over-expression, which has been associated with worse prognosis as well. The two molecules may be potent signaling partners, and based on animal models, effective EGFR and HER2 double blockade may result in improved clinical therapies and thus warrants further study in the clinical setting.

One of our most intriguing findings is the association of EGFR expression with clinical outcome. There were notable associations between EGFR expression and worse DFS and OS in patients who received systemic treatment (chemotherapy, tamoxifen, or both) but not in those who did not receive systemic therapy.

These findings have two possible explanations. First, the two groups were unbalanced in regards to a number of factors. As expected, the untreated patients tended to have tumors with more favorable clinical and biologic features (Table 4). If the event rate in this group is relatively low, the effect of EGFR expression would need even larger numbers to become evident. In a higher risk population with greater event rates, the adverse prognostic effect of EGFR would be more clearly discernable.

Alternatively, EGFR may result in poorer outcome by causing resistance to systemic treatments. In support of this, EGFR expression has been associated with resistance to hormone therapy and chemotherapy in a number of studies [9,11,43,49–51]. These two possible possibilities may also explain the significant difference in post-relapse survival between patients with EGFR-positive and EGFR-negative tumors.

Targeting EGFR therapeutically resulted in limited efficacy with the use of small molecules such as gefitinib and erlotinib [52–54], or with monoclonal antibodies such as cetuximab [55]. Reasons for this may be the complexity and redundancy of its signaling network, and

therefore, multiple points in the pathway may need to be blocked [56]. Unlike lung cancer, there is no evidence that altered forms of the receptor influence the efficacy of current therapeutics [57,58]. However, there may be activating mutations of downstream components of the signaling axis. Recent evidence shows that downstream mutations in K-RAS limits response to EGFR targeted therapies in colon and lung cancer [38–40] and warrants investigation in breast cancer. Additionally, there is a clear need for technologies that functionally or globally assess the activated status of EGFR and related downstream pathways.

This study has several limitations: It is a retrospective review of a non-randomized database collected over a long period of time. Treatment was not controlled or specified, and chemotherapy was heterogeneous, although most patients received CMF or a CMF-like regimen. However, this remains the largest and most comprehensive study of EGFR in breast cancer. It provides further insight into this so-far elusive biomarker, its effect on tumor biology, and its clinical impact.

EGFR needs to be studied as part of a robust signaling network with multiple signaling partners, intricate downstream components, and crosstalk with other pathways. Only small amounts of this receptor may need to be expressed to exert an effect on tumors. Assessment of the EGFR pathway may also need a functional assay to discern its signaling activity. This may help identify subgroups of patients in whom EGFR or related pathways play an important role, and may enable specific and effective inhibition of these active signaling elements by targeting EGFR, its signaling partners, or downstream pathways. This may be the best way to realize the promise of EGFR as a biomarker and therapeutic target, a promise that is earnestly awaited and long overdue.

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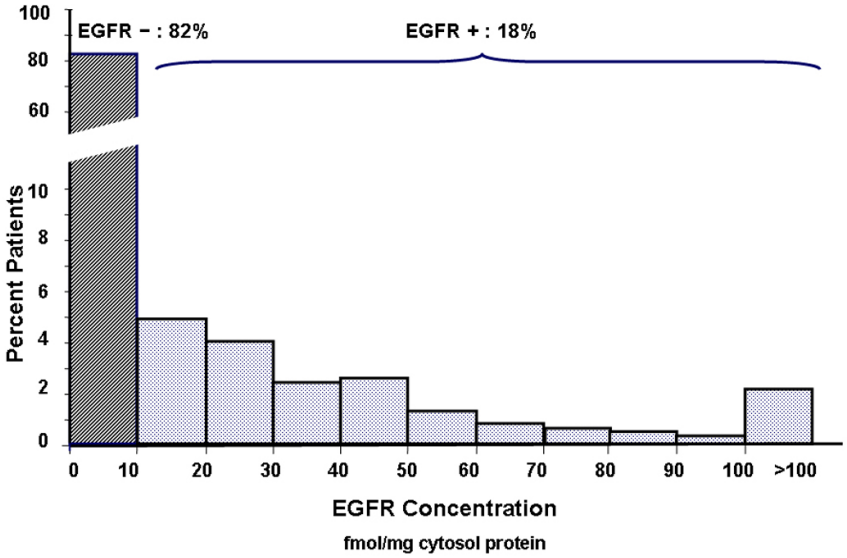

Figure 1.

EGFR concentration histogram: EGFR concentration was determined using ligand binding assay. Tumors with protein concentration of ≥10fmol/mg membrane protein were prospectively deemed positive. Of the tumors with known EGFR status, 18% (n=475) were EGFR positive and 82% (n=2092) were EGFR negative. EGFR levels of EGFR-positive tumors varied widely (10–11084 fmol/mg) but 90% had concentrations less than 100 fmol/mg.

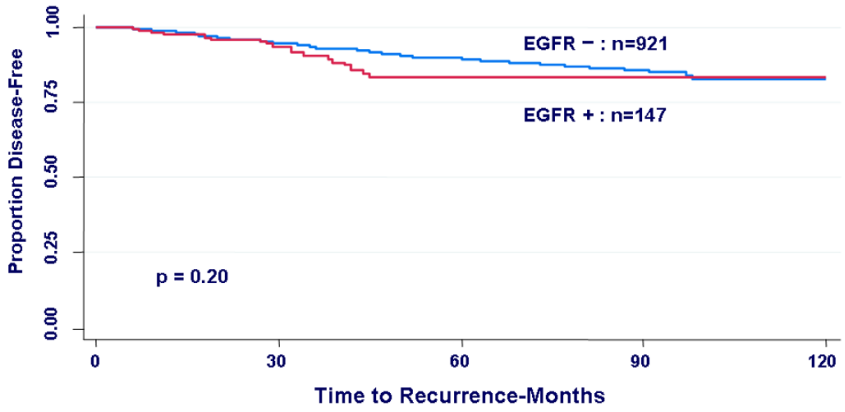

Figure 2A

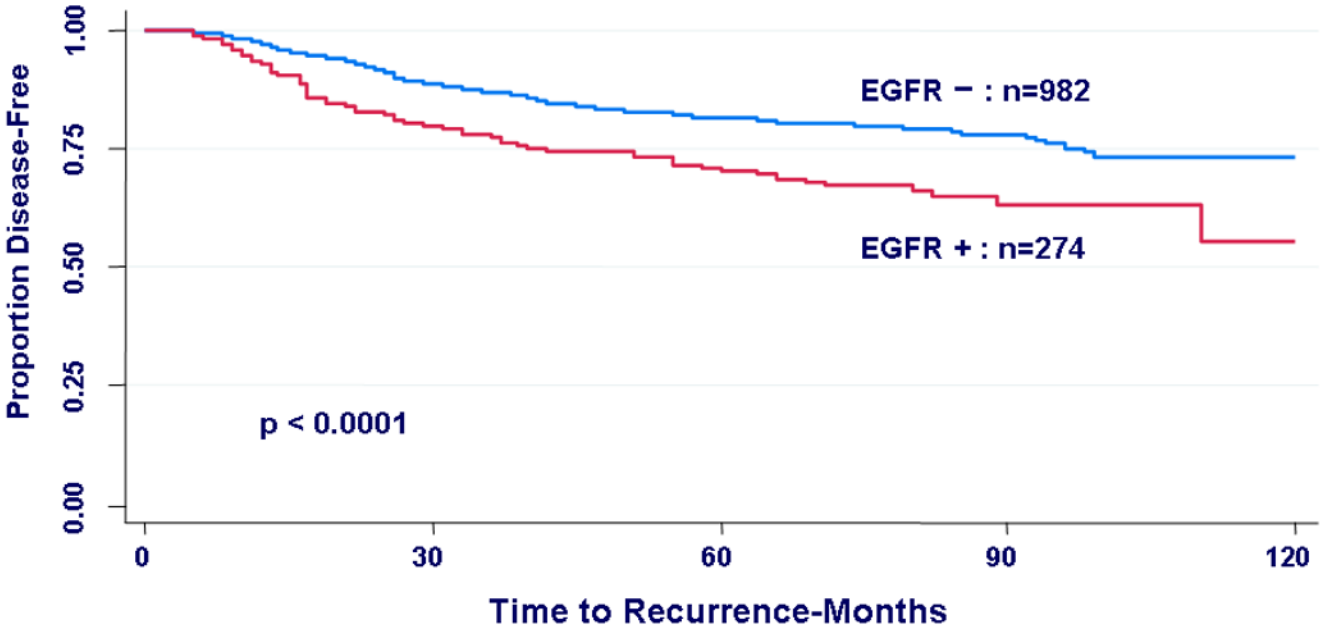

Figure 2B

Figure 2.

Kaplan-Meier curves for disease-free survival by EGFR status. **A)** In patients who did not receive any adjuvant systemic therapy (untreated patients), HR = 1.37, 95% CI (0.85 –

2.21), (p=0.20). **B)** In patients who received any form of adjuvant systemic therapy: chemotherapy, hormonal therapy, or both (treated patients), HR = 1.77, 95% CI (1.36 – 2.29), (p<0.0001).

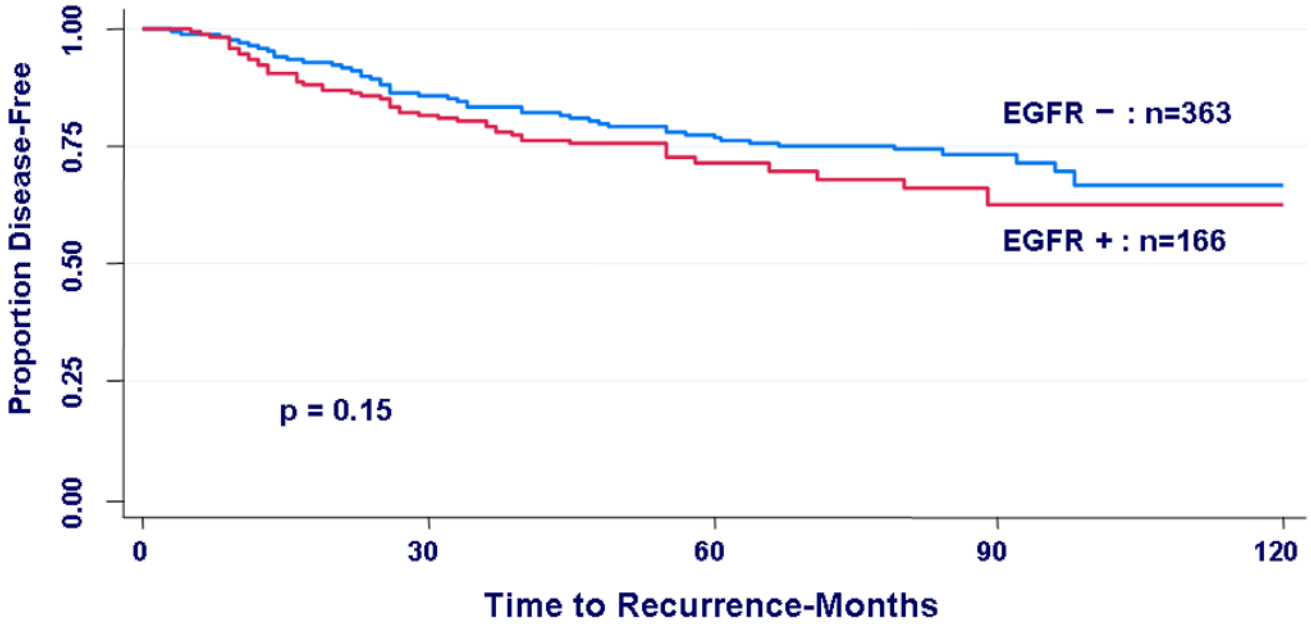

Figure 3A

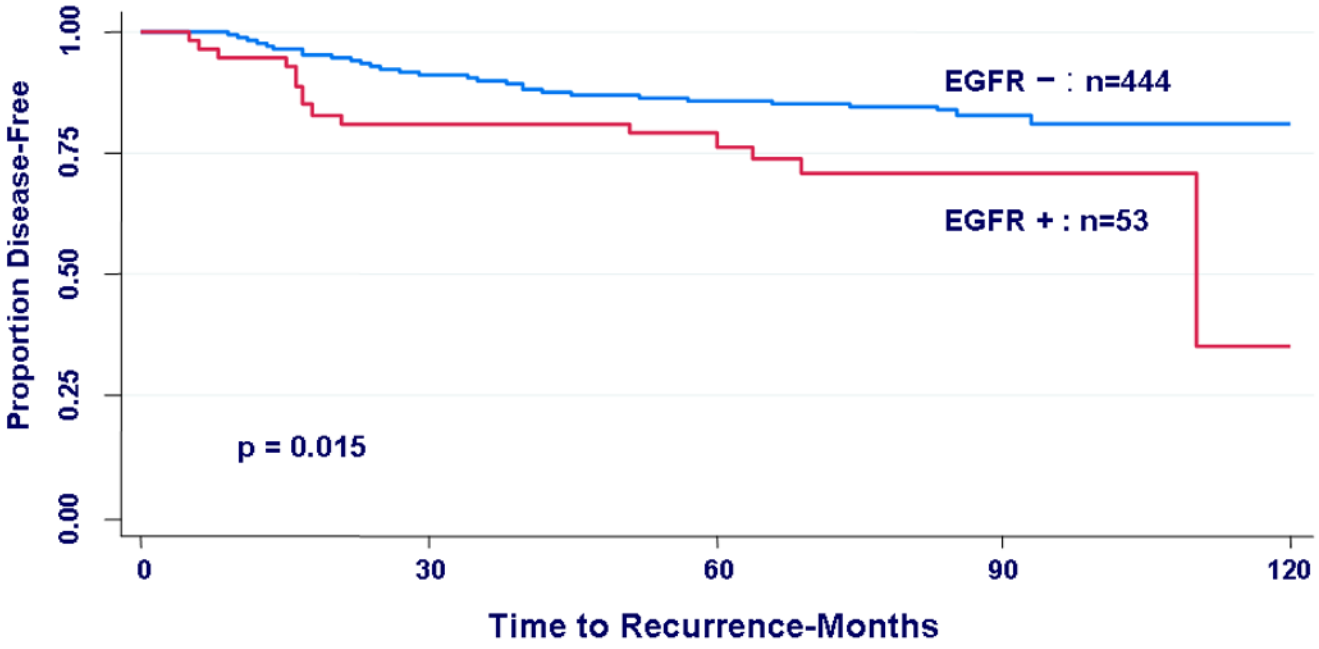

Figure 3B

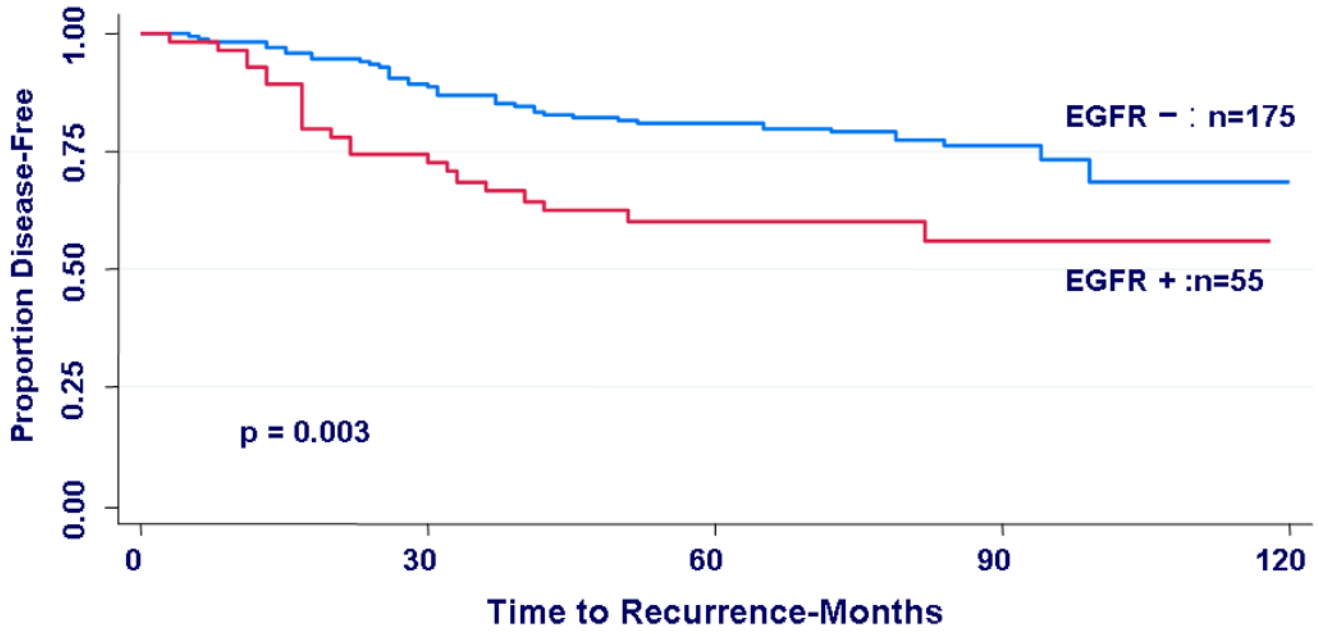

Figure 3C

Figure 3.

Kaplan-Meier curves for disease-free survival by EGFR status. **A)** In patients who received any adjuvant chemotherapy, HR = 1.30, 95% CI (0.91 – 1.86), (p=0.15). **B)** In patients who received adjuvant hormonal therapy, HR = 1.99, 95% CI (1.13 – 3.49), (p=0.015). **C)** In patients who received both chemotherapy and hormonal therapy, HR = 2.20, 95% CI (1.30 – 3.71), (p=0.003).

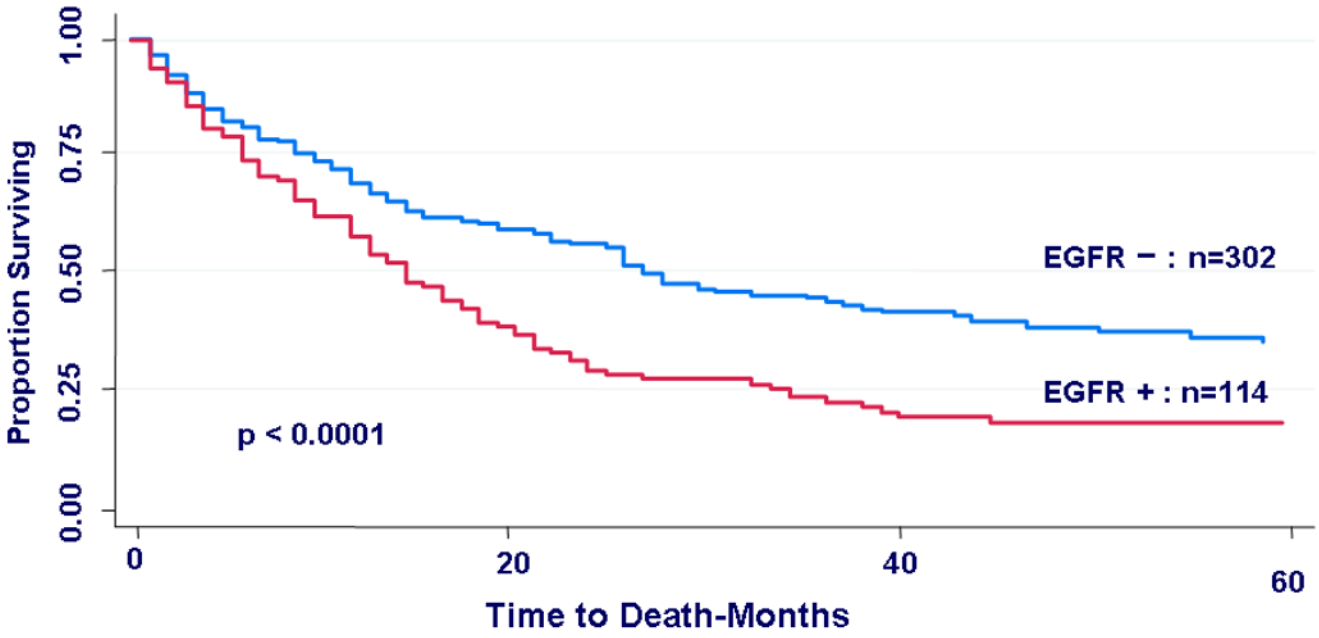

Figure 4.
Kaplan-Meier curves for post-relapse survival by EGFR status. In the subgroup that suffered tumor relapse (n=416), patients whose primary tumors were EGFR-positive had a significantly worse post-relapse survival than those with EGFR negative primary tumors, (HR = 1.72, 95% CI (1.34 – 2.20), $p < 0.0001$).

Table 1

Clinical and biological characteristics between EGFR-positive and EGFR-negative groups

Variable		EGFR +	EGFR −	p-value
Age	No. tested	473	2079	
	Median age	54	62	
	<50 yrs	40%	24%	<0.0001
	≥50 yrs	60%	76%	
Race	No. tested	401	1793	
	White	87%	92%	
	Black	9%	6%	0.0029*
	Other	3%	2%	0.1490**
Menopause status	No. tested	258	1459	
	Pre/Peri	20%	10%	<0.0001
	Post	80%	90%	
Histology	No. tested	475	2092	
	DCIS/LCIS	0	0	<0.0001
	IDC	87%	81%	
	ILC	3%	9%	
	Other	10%	10%	
Tumor size	No. tested	454	1966	
	≤2 cm	36%	54%	<0.0001
	>2cm	64%	46%	
Nodal status	No. tested	424	1839	
	0	57%	63%	
	1–3	26%	22%	0.034
	>3	17%	15%	
DNA ploidy	No. tested	469	2046	
	% diploid	32%	54%	<0.0001
	% aneuploid	68%	46%	
S-phase	No. tested	396	1855	
	Low	27%	57%	
	Intermediate	20%	21%	<0.0001
	High	53%	22%	
ER	No. tested	475	2092	
	Positive	60%	88%	<0.0001
	Negative	40%	12%	
PR	No. tested	475	2092	
	Positive	26%	65%	<0.0001
	Negative	74%	35%	
HER2	No. tested	430	1770	
	Positive	26%	16%	<0.0001
	Negative	74%	84%	

Variable		EGFR +	EGFR −	p-value
Hormone receptor groups	No. tested	475	2092	
	ER+/PR+	23%	63%	<0.0001
	ER+/PR−	37%	25%	
	ER−/PR+	3%	2%	
	ER−/PR−	37%	9%	

* This Chi-square test p-value compares the distribution of EGFR status between White and Black patients.

** This Fisher's test p-value compares the distribution of EGFR status between White and Other Race patients.

Table 2

Comparison of EGFR dataset to all patients in the database

Characteristic		EGFR data	No EGFR data
Age	Number tested	2552	52,293
	Median Age	61	62
	< 50 years	27%	25%
	≥ 50 years	73%	75%
Tumor size	Number tested	2420	50,229
	≤ 2 cm	51%	51%
	2 – 5 cm	42%	42%
	> 5 cm	7%	7%
Nodal status	Number tested	2263	48,376
	0	62%	60%
	1–3	23%	23%
	> 3	15%	18%
Tumor ploidy	Number tested	2515	4366
	% Diploid	50%	47%
	% Aneuploid	50%	53%
S-phase	Number tested	2251	3912
	Low	52%	52%
	Intermediate	20%	19%
	High	28%	28%
ER	Number tested	2567	52,298
	Positive	83%	82%
	Negative	17%	18%
PR	Number tested	2567	52,298
	Positive	58%	60%
	Negative	42%	40%
HER2	Number tested	2200	56
	Positive	18%	7%
	Negative	82%	93%
Hormone receptor groups	Number tested	2567	52,298
	ER+/PR+	56%	57%
	ER+/PR−	27%	24%
	ER−/PR+	2%	3%
	ER−/PR−	14%	15%

Table 3

Final fitted model of main effects on survival

Variable	Treated Multivariate DFS HR (95% CI)	Treated Multivariate DFS p-value	Treated Multivariate OS HR (95% CI)	Treated Multivariate OS p-value
EGFR positive vs. EGFR negative	1.66 (1.145, 2.408)	0.0074	1.98 (1.360, 2.880)	0.0004
Race: black vs. white	1.96 (1.143, 3.370)	0.0145	2.94 (1.765, 4.887)	< 0.0001
Tumor size: > 2 cm vs. ≤ 2 cm	.	.	2.08 (1.413, 3.064)	0.0002
Node positive vs. node negative	2.72 (1.875, 3.939)	< 0.0001	2.15 (1.478, 3.136)	< 0.0001
Age: ≥ 50 yrs vs. < 50 yrs	.	.	1.51 (1.039, 2.193)	0.0308

Table 4

Comparison of tumor characteristics by treatment group

		Any Treatment (n=1256)	No Treatment (n=1068)	p-value
EGFR	EGFR+	22%	14%	p < 0.0001
	EGFR−	78	86%	
Age (years)	Median Age	57	65	
	< 50 years	34%	18%	p < 0.0001
	≥ 50 years	66%	82%	
Tumor Size (cm)	> 2 cm	55%	41%	
	≤ 2 cm	45%	59%	p < 0.0001
Positive Nodes	> 3	22%	6%	
	1–3	30%	14%	p < 0.0001
	0	48%	80%	
Tumor Ploidy	Aneuploid	53%	45%	
	Diploid	47%	55%	p = 0.0002
S-phase	High	31%	22%	
	Intermediate	21%	19%	p < 0.0001
	Low	47%	58%	
ER	Negative	18%	14%	
	Positive	82%	86%	p = 0.0118
PR	Negative	42%	41%	
	Positive	58%	59%	p = 0.6068
HER2	Positive	19%	16%	
	Negative	81%	84%	p = 0.0560