EGFR amplification and PI3K pathway mutations identify a subset of breast cancers that synergistically respond to EGFR and PI3K inhibition

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Abstract # 1173

Background

Epidermal Growth Factor Receptor (EGFR) family signaling is commonly dysregulated in cancer by amplification or activating mutations. Although studies have investigated dual EGFR/PI3K inhibition in breast cancer, they have not determined biomarkers that predict success. Here, we present a subset of patients with EGFR amplification and PI3Kinase pathway mutations in breast cancer that can be synergistically targeted by dual EGFR/PI3K inhibition.

Aims

1) Investigate the frequency of EGFR amplification in breast cancer and the clinical outcomes in patients with EGFR amplification

2) Determine the frequency of coincident mutations in the PI3K pathway in tumors with EGFR amplification

3) Determine the efficacy of EGFR and PI3K targeted therapy in EGFR amplified tumor models

Methods

Frequency of *EGFR* amplification and PI3K pathway mutations (*PIK3CA, PIK3R1/2, PTEN, AKT1/2/3, TSC1/2/3*) were studied through datasets from cBioPortal and Caris Life Sciences. Triple negative breast cancer (TNBC) cell lines used include BT20 (amplified *EGFR, PIK3CA* activating mutation), MDA-MB-468 (amplified EGFR, PTEN deletion), and MDA-MB-231 (no *EGFR* or PI3K alterations). *EGFR* amplification was determined by immunoblot and fluorescent in situ hybridization, and PI3K mutations by sequencing. Signaling was determined by immunoblot, drug synergy by cell viability, cell death by propidium iodide staining, cell cycle analysis by flow cytometry, and xenograft animal study. Caris Life Science CODEai was used to evaluate real-world overall survival (OS) obtained from insurance claims and calculated from date of tumor biopsy to last contact using Kaplan-Meier estimates. Statistical significances were determined by chi-square and Mann-Whitney U test, student's t-test with 2 tailed comparisons assuming equal variance, one-way or two-way ANOVA were performed as indicated. P-values of ≤ 0.05 were considered significant.

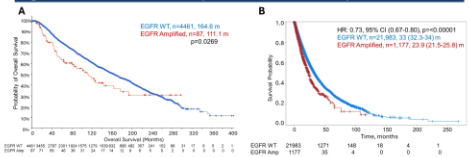
Results

Table 1: Incidence of EGFR amplification in breast cancer

	TCGA		METABRIC		MSKCC		Caris	
Breast Cancer Subtype	Samples #	EGFR Amplification (%)	Samples #	EGFR Amplification (%)	Samples #	EGFR Amplification (%)	Samples #	EGFR Amplification (%)
All Patients	816	141(17.7%)	2173	82 (2.4%)	1918	23 (1.2%)	23160	1177 (5.1%)
Histology								
IDC	489	10 (2.0%)	1660	48 (2.9%)	1473	21 (1.4%)	8411	434 (5.2%)
Mixed IDC/LC	88	0 (0%)	218	2 (0.9%)	89	0 (0%)	nd	nd
ILC	127	0 (0%)	172	1 (0.6%)	269	0 (0%)	1311	27 (2.1%)
Other Histologies	112	4 (3.6%)	123	1 (0.8%)	87	2 (2.0%)	13438	716 (5.3%)
ER and HER2 Status								
ER+	600	6 (0.8%)	1617	23 (1.4%)	1012	13 (0.9%)	10902	393 (3.4%)
ER+/HER2-	326	3 (0.9%)	1288	19 (1.4%)	1357	11 (0.8%)	9887	342 (3.8%)
ER Negative	178	9 (4.8%)	523	28 (5.4%)	327	9 (2.8%)	6312	612 (9.1%)
HER2 Positive	121	3 (2.5%)	247	9 (3.6%)	240	3 (1.3%)	1626	73 (4.5%)
ER+/HER2+	89	1 (1.1%)	108	1 (0.9%)	76	1 (1.3%)	916	21 (2.3%)
ER-/HER2+	31	2 (6.5%)	139	8 (5.8%)	79	1 (1.3%)	789	52 (7.2%)
TNBC	90	6 (6.7%)	335	19 (5.7%)	261	8 (3.2%)	6336	446 (8.4%)
Molecular Subtype								
Total Profiled	887	10 (1.7%)	1980	47 (2.4%)	nd	nd	12872	611 (4.8%)
Luminal A	397	2 (0.7%)	709	8 (0.7%)	nd	nd	1764	81 (2.8%)
Luminal B	122	2 (1.6%)	475	7 (1.5%)	nd	nd	6402	183 (2.8%)
HER2 Enriched	81	2 (3.8%)	224	12 (8.4%)	nd	nd	2233	173 (7.7%)
Basal	187	4 (3.7%)	209	17 (8.1)	nd	nd	3367	238 (6.8%)
Claudin Low	nd	nd	218	3 (1.4%)	nd	nd	nd	nd
Normal	nd	nd	148	3 (2.0%)	nd	nd	104	6 (5.8%)

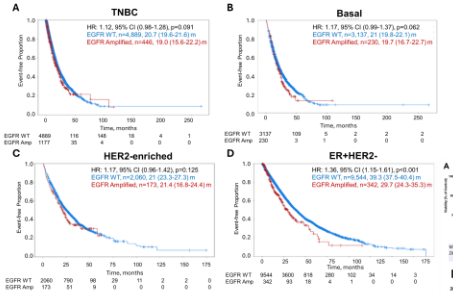
Breast cancer subtype analysis of patients in TCGA, METABRIC, MSKCC and Caris dataset with EGFR amplification. IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; nd, not done.

Figure 1: Overall Survival of patients with EGFR amplification



A) Combination of TCGA, METABRIC, MSKCC datasets to analyze overall survival (OS) of patients comparing EGFR amplification (red line) to patients without EGFR amplification (blue line). B) OS of patients from Caris dataset with EGFR amplification (red line) compared to patients without EGFR amplification (blue line).

Figure 2: Overall Survival of patients with EGFR amplification by Breast Cancer Subtype



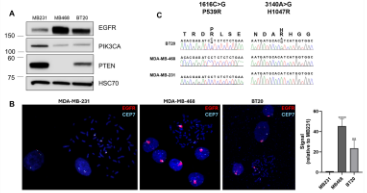
Using the Caris dataset, overall survival of breast cancer patients with EGFR amplification (red) vs WT (blue) in A) Triple negative breast cancer, B) Basal, C) HER2-enriched, or D) ER+ HER2-.

Table 2: PI3K pathway mutations in EGFR amplified breast cancer

	TCGA, METABRIC, MSKCC combined	Caris
Gene	Potential PI3KPathway Alterations (%)	Potential PI3KPathway Alterations (%)
PIK3CA	36	36.5
PIK3R1	4	4.32
PIK3R2	4	0
PIK3R3	4	Not Available
PTEN	8.3	10.5
AKT1	8.3	6.62
AKT2	6.7	0
AKT3	17.3	0
RPS6KB1	8	Not Available
RPS6KB2	4	Not Available
RPS6	4	Not Available
Patients with at least one PI3K activation	70.7	50.8

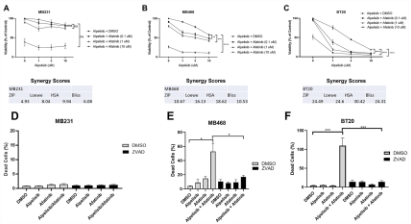
Datasets from TCGA, METABRIC and MSKCC combined showing alterations in PI3K pathway genes in 75 patients with EGFR amplification. Dataset from Caris showing alterations in PI3K pathway genes in 1,177 breast cancers with EGFR amplification.

Figure 3: EGFR protein expression and gene amplification, and PI3K mutations in MDA-MB-231, MDA-MB-468 and BT20 cells



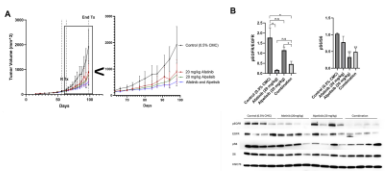
A) Western blot showing protein expression of EGFR, PIK3CA, PTEN and HSC70 (loading control) in MDA-MB-231, MDA-MB-468 and BT20 cells. B) FISH analysis showing EGFR (red) and CEP7 (aqua, control) in MDA-MB-231, MDA-MB-468 and BT20 cell lines. Average EGFR signal per nuclei was quantified using ImageJ, and statistical analysis was performed by Student's t-test, with **** indicating p<0.0001 and ** indicating p<0.01 compared to MB231. C) DNA sequencing for PIK3CA mutation status in BT20, MDA-MB-468 and MDA-MB-231 cell lines.

Figure 4: Dual EGFR/PI3K inhibition synergistically reduces cell viability, induces apoptotic cell death in TNBC with EGFR amplification and PI3K alteration



A) MDA-MB-231, B) MDA-MB-468 and C) BT20 cells all in three or more independent experiments were treated with the indicated doses for 48 hours, and then viability was determined by Promega CellTiter-Glo 2.0. Data represents the average ±SEM of the independent experiments, and statistical analysis was performed as Two-Way ANOVA, and the p value indicates interaction, where ns indicates not statistically significant, ** indicates p<0.01, *** indicates p<0.001 and **** indicates p<0.0001. The averages were also subjected to four different synergy score analyses through synergyfinder.org, where a score of >+10 indicates synergy, -10 to +10 indicates additive effects, and <-10 indicates antagonism. D) MDA-MB-231 (1 μM afatinib), E) MDA-MB-468 (3 μM alpelisib, 10 μM afatinib) or F) BT20 cells (1 μM alpelisib, 1 μM afatinib) were pretreated with 20 μM Z-VAD-FMK (ZVAD) for one hour, and then were treated with indicated drugs with continued ZVAD treatment in 1% FBS RPM1 media containing propidium iodide for 72 hours. Imaging of the cells was performed by BioTek Cytation 1, and dead cell percentage was calculated by dividing PI positive cells by the total brightfield cell count and multiplying by 100. Data represents the average ±SEM of three independent experiments. Statistical analysis was performed by Student's t-test, with *** indicating p<0.001.

Figure 5: Dual EGFR and PI3K inhibition is reduces tumor volume and inhibits EGFR and PI3K signaling



A) BT20 cells were injected subcutaneously into the mammary fat pad of nude mice. 56 days post implantation, when the average size was 100 mm^3, mice were randomized (R) to 15 mice into four groups and treated starting on day 63 post implantation (Tx) with control (0.5% carboxymethylcellulose) (black), 20 mg/kg afatinib (red), 20 mg/kg alpelisib (green), or the combination of both afatinib and alpelisib (blue) Monday through Friday for 36 days. Zoomed inset shows day 63 to 100. Statistical analysis was performed by Student's t-test, and * indicates p<0.05 when comparing afatinib and alpelisib to control on day 94 and day 98. B) After four days of treatment, four mice from each group were euthanized and the protein from the tumors was collected and immunoblotted for pEGFR (Y1068), EGFR, pS6 (S235/236), S6, and HSC70 (loading control). Data represents the average of four mice from each group ±SEM and statistical analysis was performed by Student's t-test, where ns indicates not statistically significant, * indicates p<0.05 and ** indicates p<0.01.

Summary

1) EGFR is amplified in 1-5% of breast cancers

2) Patients with EGFR amplification have a worse OS

3) EGFR amplification is more frequent in TNBC (3.7-8.4%), basal (3.7-8.1%), and HER2 enriched (3.9-7-7%) tumors

4) Dual inhibition of EGFR and PI3K synergistically induces apoptotic cell death in breast cancer models with EGFR amplification and activating PI3K mutations

5) Dual inhibition of EGFR and PI3K inhibits tumor growth in an animal model of TNBC

Conclusions

This study identifies that there is a subset of breast cancer patients with EGFR amplification with co-incident PI3K pathway mutations who are likely to respond to this combination therapy targeting the EGFR and PI3K pathway. . This ultimately could shift the paradigm for the treatment regimen in this patient population with EGFR amplification.

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