**IKZF3 amplification frequently occurs in HER2-positive breast cancer and is a potential therapeutic target**

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**Abstract**

Breast cancer is one of the leading causes of cancer death in women, and although treatment outcome has substantially improved in the past decades, advanced or metastatic breast cancers still carry a poor prognosis. Gene amplification is one of the frequent genetic alterations in cancer, and oncogene amplification may be associated with cancer aggressiveness and oncogenicity. Targeting amplified genes such as HER2 has vastly improved disease outcome and survival, and anti-HER2 therapeutics have revolutionized the standard of care in HER2 breast cancer. Besides currently known druggable gene amplifications including ERBB2 and FGFR2, other frequently amplified genes are relatively less well known for function and clinical significance. By querying four large databases from TCGA and AACR-Genie, from a total of 11,890 patients with invasive ductal breast carcinoma, we discover IKZF3, CCND1, ERBB2 to be consistently amplified across different cohorts. We further identify IKZF3 as a frequently amplified gene in breast cancer with a prevalence of 12–15% amplification rate. Interestingly, IKZF3 amplification is frequently co-amplified with ERBB2/HER2, and is also associated with worse prognosis compared to IKZF3 non-amplified cancers. Analysis of HER2 breast cancer patients treated with trastuzumab revealed decrease in both ERBB2/HER2 and IKZF3 expression. Further investigation using the DepMap for gene dependency by genome-wide CRISPR screening revealed dependence on IKZF3 in HER2 breast cancer cell lines. Our study utilized an integrative analysis of large-scale patient genomics, transcriptomics and clinical data to reveal IKZF3 as a frequently amplified gene, and suggest a potential role of IKZF3 as a druggable target for HER2 breast cancer.

**Keywords** IKZF3 · HER2 · Breast cancer

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**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| BC           | Breast cancer |
| DAVID        | Database for Annotation, Visualization, and Integrated Discovery |
| DFS          | Disease-free survival |
| GEPIA        | Gene Expression Profiling Interactive Analysis |
| GO           | Gene ontology |
| HER2         | Epidermal growth factor receptor 2 |
| IMiDs        | Immunomodulatory imide drugs |
| IPA          | Ingenuity pathway analysis |
| IDC          | Invasive ductal breast carcinoma |
| KEGG         | Kyoto Encyclopedia of Genes and Genomes |
| mBC          | Metastatic breast cancer |
| OS           | Overall survival |
| PFS          | Progression-free survival |
| PROTACs      | Proteolysis targeting chimeras |
| TCGA         | The Cancer Genome Atlas |
Introduction

Breast cancer (BC) is the most common female cancer worldwide [1]. The mortality rate of BC has persistently been high, second only to lung cancer in the United States. [2]. The outcome of BC has improved in the past two decades, largely owing to improved treatment modalities [3]. However, the prognosis of advanced BC remains poor, and even with tremendous breakthrough in novel treatment for HER2 and HR(+) metastatic breast cancer (mBC), best first-line treatment for progression-free survival is still less than 3 years and overall survival is approximately 5–6 years [4, 6], still a far cry from curing the disease. Despite a declining trend of mortality rates in BC, mBC patients still compose a huge medical burden, and treatment options for better clinical outcome remains a huge unmet need.

Since the discovery and successful development of treatments towards BC patients with HER2 amplification, research towards understanding the role of gene amplification in breast cancer rapidly intensified. Gene amplification is defined as an increase of the gene copy number in a limited region of chromosome arm [6], exerting biological effect on cell proliferation, apoptosis, genome stability, angiogenesis, invasion and metastasis [7], as well as being implicated in drug resistance [8]. In breast cancer, several amplified genes including ERBB2, EGFR, MYC, CCND1, and MDM2 has been significantly associated with higher tumor grade and aggressiveness. Amplification of ERBB2 and MYC, as well as other genes, has also been linked to decreasing survival [9]. Amplification of other oncogenes have been actively investigated for therapeutic development. Tumors with EGFR amplification have been occasionally treated with EGFR inhibitors [10], and FGFR-amplified cholangiocarcinoma can be treated with FGFR inhibitors [11]. However, many other common amplifications in tumors are not yet well understood, which could be of huge interest for both oncology and drug development fields.

In our study, we sought to discover common gene amplification events that could be of clinical interest in breast cancer. Our study revealed IKZF3 amplification as a commonly occurred, but relatively underreported gene amplification. IKZF3 encodes for IKAROS Family Zinc Finger three, also named as Aiolos. The IKAROS family includes five members (Ikaros (IKZF1), Helios (IKZF2), Aiolos (IKZF3), Eos (IKZF4), Pegasus (IKZF5)), which are zinc finger transcription factors that modulate lymphocyte cell function and maturation [12]. Ikaros (IKZF1) and Aiolos (IKZF3) have been established for targeted destruction by imides (thalidomide, lenalidomide, pomalidomide) to exert their anti-cancer activities in multiple myeloma [13]. Very few studies have examined the role of IKZF3 in breast cancer. IKZF3 was among a set of genes associated with breast cancer endocrine resistance in the SEER database [14]. Another study using comprehensive genetic profiling on 54 HER2-amplified breast cancer samples identified IKZF3 among other genes to be frequently co-amplified in the ERBB2/HER2 amplicon[15]. However, no large-scale clinical analysis has yet directly evaluated the role of IKZF3 in breast cancer. In our study, we investigate the potential role of IKZF3 in breast cancer and clinical significance. Our findings suggest IKZF3 as a potential target for HER2-expressing breast cancer for academic research and therapeutic development.

Methods

Oncomine database

We queried the Oncomine database (www.oncomine.org) [16], an online web-based data-mining platform on cancer samples for of IKZF3 in different clinical cancer specimens and corresponding normal controls. The search contents and cut-off were as follows. Keywords: IKZF3, primary filter, cancer vs. normal; cancer type, BC, the absolute value of log2 fold change > 1.5, P < 0.05; and gene rank, 10%. The P-value was calculated using the Student’s t test.

GEPIA database

We investigated the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/) [17] for tumor versus normal differential expression analysis and further analysis based on The Cancer Genome Atlas (TCGA) and the genotype–tissue expression data.

TCGA database and cBioPortal

We queried cBioPortal for Cancer Genomics (http://cibioportal.org) for analyzing complex and multifactorial cancer genomic data from TCGA [18]. We included two TCGA datasets of BC, namely, “METABRIC Breast cancer cohort (1865 breast invasive ductal carcinoma cases)”, “Breast Invasive Carcinoma, TCGA, PanCancer Atlas (780 breast invasive ductal carcinoma cases)” and two AACR-GENIE databases (accessed through the AACR-GENIE cBioPortal, https://genie.cbioportal.org/): “Metastatic Breast Cancer: 2013–16 (856 cases) “, “GENIE Cohort v10.0-public (8389 breast invasive ductal carcinoma cases)”. The OncoPrint, survival tabs and other web-based analysis were applied pursuant to cBioPortal instructions.
String database and DAVID

Transcriptional profiling pathway analysis was performed by Database for Annotation, Visualization, and Integrated Discovery (DAVID) (https://david.ncifcrf.gov) [19, 20] and through the use of IPA (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis) to perform Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. The human genome was selected as the background parameter, and a $P < 0.05$ was considered statistically significant.

DepMap analysis

Genome-wide CRISPR dependency data (21Q2 Achilles_gene_effect file) were analyzed from Broad Institute DepMap web portal (https://depmap.org/portal/download/).

Results

IKZF3 amplification occurs frequently in breast cancer with a prevalence of 12–15%

To systemically investigate commonly amplified genes in human breast cancer, we queried four major databases deposited in cBioPortal [18], including the Breast Invasive Carcinoma TCGA-PanCancer data [21], the AACC Project GENIE Consortium [22], the Metastatic Breast Cancer DFCI-Profile cohort [23, 24], and the TCGA-METABRIC cohort [25, 26].

We confined our query to specifically include patients with invasive ductal breast carcinoma (IDC). From the aforementioned databases in cBioPortal, the frequency of amplified genes was listed and ranked in each database. We used an arbitrary cut-off of 10% frequency for gene amplification, i.e., only genes with a ≥ 10% amplification frequency were selected for further analysis. We discovered that there was a vast difference between databases of numbers of gene amplification reported with ≥ 10% frequency. In the TCGA-METABRIC and PanCancer cohorts, the numbers of amplified genes with ≥ 10% frequency were 1579 and 476, respectively, while in AACR genie and the DFCI cohort (which was curated under the AACR-genie program), the numbers of amplified genes were 19 and 3, respectively. This may be related to the threshold of gene amplification reporting, as AACE-genie guidelines state only CNAs with a ploidy of five or higher are reported as gene amplification [27]. Nevertheless, the three genes from the DFCI cohort (IKZF3, CCND1, ERBB2) were consistently reported as amplified in all the 4 cohorts (Fig. 1A, Table 1). An additional 11 genes (COL22A1, FGFRI, SETDB1, FGF19, UBR5, FGF4, NSD3, MYC, TG, PDE4DIP, FGF3) were reported as amplified in the AACR-genie, TCGA-METABRIC and TCGA-Pancancer cohorts but not in the DFCI cohort.

Therefore, we concluded that IKZF3, CCND1, ERBB2 was consistently reported as amplified in human breast cancers, regardless of different cut-off criterion.

Further analysis into Table 1 revealed several interesting observations: the 11q13.3 arm harbored CCND1, FGF3, FGF4, FGF19 that were consistently amplified in the three cohorts (TCGA-METABRIC, TCGA-Pancancer, AACR-genie). Other hotspots included chromosome 8, (FGFR1, NSD3 on 8p1.1.23, and MYC, COL22A1, TG, UBR5 in 8q24), chromosome 1 (PDE4DIP, SETDB1 on 1q21.3), and chromosome 17 (ERBB2, IKZF3 on 17q12). The three genes IKZF3, CCND1, ERBB2 were consistently amplified across all four cohorts analyzed. CCND1 encodes for cyclin D1, which is well characterized for its role in cell cycle. The role of cyclin D1 amplification in breast cancer has been well investigated and associated with a poor outcome in the METABRIC cohort [28]. Since cyclin D1 is an important element of the cell cycle, CDK4/6 inhibitors are theoretically effective in these group of patients. CDK4/6 inhibitors in luminal breast cancers have revolutionized the standard of care; however, amplification of cyclin D1 was not associated with a differential response to palbociclib in the PALOMA-1 trial [29]. ERBB2 encodes for HER2, and anti-HER2 therapeutics have drastically altered the nature course of HER2 amplification breast cancers [30, 31]. Compared to ERBB2 and CCND1, the role of IKZF3 has never been studied in a large-scale breast cancer patient cohort. Therefore, we elected to further investigate the role of IKZF3 amplification in clinical outcomes of breast cancer. To visualize the relative location of IKZF3 to ERBB2, we illustrated the proposed ERBB2 amplicon [15] on chromosome 17 using the Genome Data Viewer (https://www.ncbi.nlm.nih.gov/gv/gd/) (Fig. 1B). Since IKZF3 belonged to the Ikaros transcriptional family with five members (IKZF1/2/3/4/5), we investigated the global expression levels in human cancers. Using the Oncomine database [16], we compared the expression levels between various human cancers. In general, only IKZF1 was more differentially expressed in breast cancer, while IKZF3 was differentially expressed in lymphoma and leukemia (Fig. 1C). Focusing on the BRCA cohort in TCGA, we observed that IKZF3 was generally underexpressed in comparison to the other members of the Ikaros family (Fig. 1D, E).

Our findings suggest that IKZF3 in general is not highly expressed in breast cancer, which suggests amplification of IKZF3 might have a more substantial effect due greater fold changes in expression.

IKZF3 amplification is highly associated with HER2 amplification and with worse outcome in breast cancer

To further delineate the role of IKZF3 in the clinical outcome of breast cancer patients, we performed in-depth analysis of the METABRIC database. Of the 1865 patients with
Fig. 1 IKZF3 amplification occurs frequently in breast cancer with a prevalence of 12–15%. **A** Venn diagram of overlapping amplified genes across the four cohorts of breast cancer. **B** ERBB2 ampli-con and surrounding genes including IKZF3 on chromosome 17. **C** Oncomine family illustration of IKZF1-5 gene expression profiles. **D** and **E** IKZF1-5 gene expression comparison between normal and tumor from TCGA database, BRCA cohort.
IDC, 1660 patients had CNV analysis, in which 258 patients (15.5%) were classified as IKZF3 amplification (IKZF3-amp) and 1402 (84.5%) as non-amplified (IKZF3 non-amp). Overall survival was significantly shorter for IKZF-amp (median 96.9 months, 95% CI: 79.97–124.13) versus IKZF3 non-amp (median 163.7 months, 95% CI: 152.07–174.80, logrank test p-value 0.000000729) (Fig. 2A). IKZF3-amp patients had several clinical features significantly different compared to IKZF3 non-amp patients (summarized in Supplemental 1: Table S1). These included: higher rates of HER2-positive status, lower rates of ER/PR positivity, younger age, higher rates of receiving chemotherapy, more advanced stages (higher proportion of stages III–IV), higher rates of mastectomy. Interestingly, 79.22% of IKZF3-amp patients were HER2 status positive (183/258), while an overwhelming 97.24% were HER2 negative in the IKZF3 non-amp group (Fig. 2B). ERBB2 was the top differentially expressed gene when compared between IKZF3-amp and non-amp (Fig. 2B). A total of 31 genes were highly differentially expressed in the IKZF3-amp group of more than twofold (log ratio ≥ 1) (Supplemental Table S2). In this group, ERBB2, GRB7, PGAP3, PPP1R1B, MIEN1, PNMT, FBXL20, CDK12 were all on 17q12, suggesting a linkage disequilibrium relationship with IKZF3. For instance, expression of GRB7, the gene adjacent to ERBB2, has been reported to be highly correlated with HER2 amplification status [32].

To further confirm this observation, we pooled the TCGA-PanCancer cohort and the TCGA-metabric cohort, resulting in a total of 360 patients with IKZF3 amplification and 2283 patients without. Overall survival was still significantly shorter for IKZF-amp (median 101.3 months, 95% CI: 87.10–132.20) versus IKZF3 non-amp (median 163.2 months, 95% CI: 152.07–174.50, logrank test p-value 0.00000061) (Fig. 2d). Since most of the samples were early breast cancer (>95% stage I or II), surgery was the mainstay treatment. The relapse free survival still showed a significant benefit in IKZF3 non-amp patients (Fig. 2E). To test whether IKZF-amp would have an adverse impact on survival independently of HER2 amplification, we compared patients with and without HER2 amplification in the IKZF3-amp subgroup. There was a trend favoring patients without Her2 amplification with better overall survival, although the difference was not statistically significant (HER2 amp vs HER2 non-amp, overall survival 87.1 months versus 129.2 months, log rank $P=0.0751$) (Fig. 2F).

**IKZF3 expression is decreased after anti-HER2 treatment**

Our studies suggest a possible role of IKZF3 in HER2 (+) breast cancer treatment. We then investigated whether the expression of IKZF3 would be modulated by anti-HER2 treatment. With an aim to investigate the changes of IKZF3 in real-world patient-based studies, we queried the GEO dataset GSE114082. This study quantified RNA-seq expression levels before and after a single dose of trastuzumab in 17 HER2-positive breast cancer patients [33]. Interestingly, both IKZF3 and ERBB2 major transcript levels decreased after treatment of anti-HER2 trastuzumab (Fig. 3A). We then performed pathway analysis using the ingenuity pathway analysis (IPA) software. RNA-seq analysis of the dataset GSE114082 revealed top enriched pathways: intrinsic prothrombin activating pathway, cytokine signaling, atherosclerosis signaling, serotonin signaling, and LXR/RXR signaling (Fig. 3B). We then analyzed differentially expressed genes with DAVID (with filter options selected: “OMIM_DISEASE “, “GOTERM_BP_DIRECT”, “GOTERM_CC_DIRECT”, “GOTERM_MF_DIRECT”, “BIOCARTA”, “KEGG_PATHWAY “), in which top ranked pathways included: Olfactory transduction, Intrinsic Prothrombin Activation Pathway, ABC transporters, G-protein coupled serotonin receptor activity, Chemical carcinogenesis (Table 2). Interestingly, intrinsic pathway of coagulation was enriched in both IPA and DAVID pathway analysis approaches. Differentially expressed genes in this pathway included F8, PROC, F9, F10, F12, F11, COL4A3, KNG1, which we confirmed were elevated from around threefold to 7.6 fold after treatment by trastuzumab, with the exception of F12 and PROC gene which were downregulated (Fig. 3C). To summarize, we discovered that IKZF3 expression decreased synchronously with ERBB2 after trastuzumab treatment. This suggested that HER2- amplification clones that also co-expressed (or co-amplified) IKZF3 might be eradicated after trastuzumab treatment. Our data also suggested that treatment of trastuzumab, and decrease of HER2/IKZF3, was associated with modification of several transcriptional pathways including the coagulation pathway.

**DepMap analysis on effect of IKZF3 gene disruption suggest dependence on IKZF3 in HER2 and triple-negative breast cancer cell lines**

To further analyze whether breast cancer cells, especially HER2-amplified cells, exert dependence on IKZF3, we queried the DepMap Portal (https://depmap.org/portal/depmap/) [34]. By interrogating the CRISPR DepMap 21Q3 Chronos dataset, focusing on ductal breast cancer cell lines, we identified cell lines that demonstrated dependency on IKZF3 (Fig. 4A). In this analysis, each cell line was tested for their dependence to IKZF3 by a genome-wide CRISPR knockout screen. Interestingly, cell lines showing mild IKZF3 dependency (Gene effect score < −0.2) were exclusively TNBC (triple-negative breast cancer) (HCC1143, MDAMB157, SUM1315MO2) and HER2 amplification (UACC893, HCC202, HCC1419) cell lines. As a comparison, we
also examined ERBB2, which showed dramatically more cell lines dependency and markedly stronger dependency (Fig. 4B) However, cell lines dependent on ERBB2 included not only TNBC and HER2 BC cell lines, but also HR(+) cell lines (T47D, ZR751, and others) demonstrating that ERBB2 is a strong oncogenic driver that elicits significant oncogene addiction. This analysis suggested that HER2 and TNBC breast cancer cell lines are more likely to be dependent on IKZF3 signaling.

**Discussion**

The development of anti-HER2 therapeutics in HER2 (+) breast cancer is one of the most dramatic breakthroughs in oncology development. The landmark clinical trials N9831 and B-31 demonstrated unprecedented efficacy with anti-HER2 monoclonal antibodies that improved disease-free survival in early HER2 (+) breast cancers [35]. Equally impressive results were demonstrated by trastuzumab [36] in HER2 (+) metastatic breast cancer, followed by the groundbreaking discovery that addition of pertuzumab to trastuzumab further prolongs survival in the seminal CLEOPATRA trial [31]. The CLEOPATRA trial reported a stunning 56-month overall survival in newly diagnosed HER2 (+) metastatic breast cancers when started on pertuzumab, trastuzumab and docetaxel, one of the longest overall survival for metastatic cancers. The most newest addition in the anti-HER2 armory is the anti-HER2 antibody drug conjugate (ADC) DS-8201, which significantly extended the progression-free survival in second-line treatment for HER2(+) metastatic breast cancer [37]. Reflecting on the progress made in the HER2 (+) breast cancer field, with 6-year disease-free survival at 91% for early cancers [38, 39] and overall survival at 56 months for metastatic cancers [31], which can possibly be further extended in the future [by current ongoing trials, including the first-line DESTINY-Breast09 (NCT04784715)], our views on the role of HER2 oncogene has evolved. HER2 amplification traditionally has been shown to confer aggressiveness in cancer [40] and projected a dismal prognosis. Nowadays with the plethora of anti-HER2 therapeutics, the outcome of HER2 (+) breast cancers have drastically improved [41]. This suggests that HER2 (+) breast cancers are highly addicted to HER2 driven oncogene signaling, and effective targeting of this pathway could lead to effective cancer killing abilities. However, even with the wave of anti-HER2 therapeutics, metastatic HER2 breast cancer is still ultimately deadly for the majority of patients. In the end-of-trial analysis of the CLEOPATRA study, only 37% remained surviving in the dual HER2-blockade group at 8 years [4]. Although this is an impressive feature in metastatic cancers, there remains potential for improvement in efficacy to treat this deadly disease.

In our study, we identified a high prevalence of IKZF3 amplification (12–15%), and high concordance rates with ERBB2/HER2 amplification. As analyzed in the results section, the amplification of IKZF3 was independently associated with worse survival. The high concordance rate of IKZF3 and HER2 amplification could explain the similarities of IKZF3 amplification and HER2 amplification breast cancers. Interestingly, ~80% of IKZF3 amplification cancers had HER2 amplification, while conversely IKZF3 non-amplification tumors were almost 100% HER2 non-amplified. This suggests that an IKZF3 amplification event could occur independently of HER2 instead of being co-amplified as a linkage event. Sircoulomb et al. profiled 54 HER2-amplified breast cancer samples and identified genes that were co-amplified or co-expressed with HER2 amplification [15]. The authors identified the span of ERBB2-C17orf37-GRB7 adjacent genes as a minimally linked

| Genes   | Chromosome | Abc (%) | PanCancer (%) | Genie (%) | DFCI (%) |
|---------|------------|---------|---------------|-----------|---------|
| CCND1   | 11q13.3    | 16.30   | 14.20         | 16.20     | 13.70   |
| COL22A1 | 8q24.23-q24.3 | 24.20   | 12.90         | 21.90     |         |
| ERBB2   | 17q12      | 17.80   | 13.50         | 13.80     | 13.60   |
| FGFI9   | 11q13.3    | 16.30   | 13.80         | 16.10     |         |
| FGFI3   | 11q13.3    | 15.60   | 13.80         | 15.30     |         |
| FGFI4   | 11q13.3    | 15.70   | 13.80         | 15.70     |         |
| FGFR1   | 8p11.23    | 13.80   | 11.50         | 11.50     | 8.80    |
| IKZF3   | 17q12-q21.1| 15.50   | 13.30         | 13.50     | 12.40   |
| MYC     | 8q24.21    | 28.60   | 18.50         | 10.40     | 5.50    |
| NSD3    | 8p11.23    | 14.20   | 11.80         | 11.50     |         |
| PDE4DIP | 1q21.2     | 15.50   | 10.20         | 13.90     |         |
| SETDB1  | 1q21.3     | 18.30   | 10.20         | 16.50     |         |
| TG      | 8q24.22    | 25.40   | 13.70         | 22.50     |         |
| UBR5    | 8q22.3     | 23.70   | 12.90         | 21.40     |         |
**Fig. 2** IKZF3 amplification is highly associated with HER2 amplification and with worse outcome in breast cancer. **A** Overall survival comparison between IKZF amplification and non-amplification cancers in the METABRIC cohort. **B** HER2 positivity in IKZF-amplified and non-amplified cancers. **C** ERBB2 expression in IKZF-amplified and non-amplified cancers. **D** Overall survival comparison between IKZF amplification and non-amplification cancers in the METABRIC and PanCancer cohorts. **E** Relapse free survival comparison between IKZF amplification and non-amplification cancers in the METABRIC and PanCancer cohorts. **F** Kaplan–Meier survival curve of HER2 positive and negative in IKZF3 amplification cancers, log rank $P = 0.0751$.
Fig. 3  IKZF3 expression is decreased after anti-HER2 treatment. A IKZF3 and ERBB2 levels fold change after trastuzumab treatment. two major transcripts for each IKZF3 and ERBB2 were shown to be decreased in the GEO cohort. B The top 18 canonical pathways of data-set GSE114082 identified by the Ingenuity Pathway Analysis (IPA) are listed here. The left y-axis represent pathways ranked by -log(P-value) in x-axis. Threshold was set to -log(P-value)> 1.3. P-value was calculated by right-tailed Fisher’s exact test determining the length of bars. A cutoff at |log2(fold change)|> 2 was set to distinguish significantly differentially expressed genes. The z-score was used to compare the uploaded dataset with the canonical pathway patterns. Orange bars: positive z-score and predicted up-regulation of the pathway; gray bars: no activity pattern available. IL, interleukin; LXR/RXR, liver X receptor/retinoid X receptor; FXR/RXR, the farnesoid X receptor/retinoid X receptor. C Differentially expressed genes fold change in the Intrinsic Prothrombin Activation Pathway in DAVID.
17q12-q21 amplicon, and mentioned IKZF3 as a frequent co-amplified gene at the telomeric border of the amplicon. The findings of the study provide invaluable context that is complemented by our study to further understand the genetics of IKZF3 and ERBB2. As expected, the ERBB2 amplicon has heterogeneity between different groups (such as ER+ versus ER- [15]). Another study proposed TCAP, PNMT, PGAP3, ERBB2, MIEN1 and GRB7 as constituents of the ERBB2 amplicon [42]. As shown in Fig. 1b, IKZF3 is immediately proximal to the proposed ERBB2 amplicon, which could potentially explain the high frequency of co-amplification with ERBB2. However, this raises an interesting question: are the genes in the ERBB2 amplicon always co-amplified with ERBB2? In the METABRIC and PanCancer cohorts, we did observe co-amplification of the six genes in the ERBB2 amplicon, but not in the AACR datasets. The method and criterion for amplification calling is different between TCGA and AACR, which could explain this discrepancy between datasets. However, the fact that IKZF3 was consistently reported as amplified across all 4 cohorts suggest that IKZF3 was tightly linked to ERBB2. The fact that IKZF3 amplification occurred in ~20% cases without ERBB2 amplification suggested the possibility that IKZF3 could be a distinct amplicon of its own.

The role of IKZF1 and IKZF3 was shown to play a major role in the tumor killing mechanism of the Immunomodulatory imide drugs (IMiDs) when used in multiple myeloma [43, 44]. This groundbreaking finding not only uncovered the long sought-for mechanism of thalidomide in multiple myeloma [45], but also elucidated the IKZF1/3-CRBN degradation pathway. CRBN is now one of the most commonly used E3 ligase for Proteolysis targeting chimeras (PROTACs) design [46, 47]. IMiDs exert their tumor killing properties by facilitating CRL4 polyubiquitination and, thus, depletion of the transcription factors IKZF1 and IKZF3, demonstrating significant cell death in multiple myeloma [48]. This finding partially led to the current development of PROTACs, a rapidly emerging field of novel anti-cancer therapeutics [49]. Our studies strongly implicate that IMiDs could possibly play a role in IKZF3-amplified cancers. To date, this has not been tested in human, with scarce preclinical studies being reported [50–53]. This hypothesis requires a prerequisite that HER2-positive breast cancers are dependent on IKZF3 signaling, which currently has not been proven. Using the DepMap genome-wide CRISPR screen, we observed that HER2 (+) breast cancer cells are dependent on IKZF3 signaling, although the dependency is not as robust as strong oncogenic drivers such as ERBB2. Current studies on IKZF3 signaling mostly conclude on its role in lymphocyte activity and in hematologic oncogenicity [54, 55]. Further gain- or loss-of-function of IKZF3 in breast

| Table 2 | Abc |
|------------------|-----------------|-----------------|-----------------|-----------------|
| Enrichment score | Term | Count | P-Value | FDR | Category |
| 85.31 | Olfactory transduction | 187 | 0.000 | 0.000 | KEGG_PATHWAY |
| 2.72 | Intrinsic prothrombin activation pathway | 8 | 0.000 | 0.028 | BIOCARTA |
| 2.26 | ABC transporters | 9 | 0.022 | 0.374 | KEGG_PATHWAY |
| 2.01 | G-protein-coupled serotonin receptor activity | 7 | 0.006 | 0.548 | GOTERM_MF_DIRECT |
| 1.72 | Chemical carcinogenesis | 17 | 0.001 | 0.060 | KEGG_PATHWAY |

Fig. 4 DepMap analysis of the dependency of ductal breast cancer cell lines in CRISPR databases. **A** Dependency score of ductal breast cancer cell lines dependent to IKZF3 using DepMap. **B** Dependency score of ductal breast cancer cell lines dependent to ERBB2 using DepMap. CRISPR databases (DepMap 21Q3), X-axis: dependency scores.
cancer cells will shed light on whether IKZF3 amplification is a driver oncogene in breast cancer biology.

Our study has several limitations. We did not perform actual mechanistic studies such as transcriptional or proteomic profiling of cells with IKZF3 disruption. Our study relies on comparing insights derived from large-scale, actual patient-derived samples and extracting genomic insights that are consistent across multiple datasets. Another shortcoming is the lack of further validation of the novel discovered pathways, such as the coagulation pathway enrichment that resulted from trastuzumab treatment. In the study GSE114082, 17 breast cancer patients were treated with one dose of trastuzumab and gene expression profiles were compared before and after trastuzumab treatment [33]. It may be argued whether one dose of trastuzumab treatment reflected the actual changes of long-term anti-HER2 treatment in HER2 breast cancers. Nevertheless, the strength of our study lies within the large patient numbers of TCGA and AACR-Genie cohorts and the clinical significance of studying real-world human data. Another interesting observation is the potential pitfalls and limitations of cross-platform sequencing. For instance, in cBioPortal, three cohorts from MSK are deposited [56–58]. However, these three do not have IKZF3 CNV data, as the panel sequenced (e.g., IMPACT 468) does not contain IKZF3 (only IKZF1). These findings highlight the caveats of using real-world studies, where each dataset have their own limitations that preclude a robust conclusion.

Conclusions
In this study, we report a high prevalence of IKZF3 amplification that co-exists frequently.

With ERBB2/HER2 amplification in breast cancer. We analyze the clinical features of IKZF3 amplification, and investigate its role in HER2 breast cancer. The protein degradation mediated by IMiDs on IKZF3 may potentially be exploited as a novel approach in HER2 breast cancer. Further mechanistic studies will confirm this hypothesis for a possible approach towards development of novel treatment agents.

Supplementary Information
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Author contributions
CIY, CIS, CYL, JIL: data collection, analysis and curation. CIY, CYL, JIL: drafting of the manuscript. CYL, TCC, CCH, LMT, JIL: conceptualization of the project. All authors read and approved the final manuscript.

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Declarations
Conflict of interest
The authors declare that they have no conflict of interest.

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