HER kinase inhibition in patients with HER2- and HER3-mutant cancers

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Somatic mutations of ERBB2 and ERBB3 (which encode HER2 and HER3, respectively) are found in a wide range of cancers. Preclinical modelling suggests that a subset of these mutations lead to constitutive HER2 activation, but most remain biologically uncharacterized. Here we define the biological and therapeutic importance of known oncogenic HER2 and HER3 mutations and variants of unknown biological importance by conducting a multi–histology, genomically selected, 'basket' trial using the pan–HER kinase inhibitor neratinib (SUMMIT; clinicaltrial.gov identifier NCT01953926). Efficacy in HER2–mutant cancers varied as a function of both tumour type and mutant allele to a degree not predicted by preclinical models, with the greatest activity seen in breast, cervical and biliary cancers and with tumours that contain kinase domain missense mutations. This study demonstrates how a molecularly driven clinical trial can be used to refine our biological understanding of both characterized and new genomic alterations with potential broad applicability for advancing the paradigm of genome-driven oncology.

Genomic profiling of human cancers has identified recurrent somatic mutations of HER2 (encoded by ERBB2) and HER3 (ERBB3), typically occurring in the absence of gene amplification1–3. Mutations in HER2 are clustered in the extracellular, transmembrane and kinase domains. Unlike other mutant oncoproteins, such as BRAF or KRAS, no single mutant allele predominates and the precise distribution of mutations varies by tumour type1. By contrast, HER3 mutations cluster primarily in the extracellular domain and to a lesser extent in the kinase domain. Although HER2 and HER3 mutations are found in a wide variety of cancers, their overall prevalence does not exceed 10% in any individual tumour type, and the rate is more typically less than 5% for HER2 and less than 1% for HER3.

Biological modelling has yielded conflicting findings as to the functional consequences of HER2 and HER3 mutations. Substantial data suggest that a subset of these mutations induce ligand-independent constitutive HER2 receptor signalling and promote oncogenesis4–7. The mechanism of these oncogenic effects seems to differ by variant, with some causing enhanced HER2 kinase activity and others causing receptor dimerization8,9. Mutations in HER3, which in its wild-type configuration has impaired kinase function, seem to rely on wild-type HER2 to exert its oncogenic effects6. Most preclinical data that explore the functional consequences of HER2 and HER3 mutations have been generated using engineered models that overexpress the mutation, and thus the results may be confounded by the known oncogenic effects of HER2 overexpression. Further enforcing the potential importance of this confounding variable, models of HER2 mutation generated by gene-editing techniques have failed to demonstrate a malignant phenotype in the absence of mutations in other oncoproteins such as PIK3CA9.

Given the considerable diversity of HER2 and HER3 mutations, as well as the challenge of generating preclinical models that recreate their true biology in human cancers, we sought to define the therapeutic importance of HER2 and HER3 mutations by conducting SUMMIT—a global, multicentre, multi-histology basket trial in patients with tumours that contain these mutations (Extended Data Fig. 1). Patients were treated with neratinib, an irreversible pan-HER tyrosine kinase inhibitor, which potently inhibits the growth of HER2-mutant tumours in preclinical models5. Tumour tissue and plasma were collected to facilitate the detailed genomic characterization of patients. Here we present the results of this study, with a focus on the insights it provides into the biological and therapeutic importance of HER2 and HER3 mutations in patients with cancer.

Patient and mutation characteristics

Baseline patient demographics are shown in Table 1 and Extended Data Table 1. In total, 141 patients (125 with HER2-mutant tumours, 16 with HER3-mutant tumours) received neratinib treatment. These patients were diagnosed with 1 out of 21 unique cancer types, the most common being breast, lung, bladder and colorectal cancer (61% of patients treated). As has been seen in other basket studies10,11, we identified and enrolled several orphan tumour types including cancers of the biliary tract, salivary gland, small bowel and vagina, as well as extramammary Paget’s disease (in aggregate, 13% of all patients). Patients tended to be heavily pretreated with approximately half having received at least three previous lines of systemic therapy.

Enrolled patients had 31 unique HER2 and 11 unique HER3 mutations (Extended Data Fig. 2). The most frequent HER2 mutations

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were S310, L755, Y772_A775dup and Y777L alleles. The HER2 kinase domain was most commonly mutated (66%), followed by the extracellular (26%) and transmembrane/juxtamembrane (8%) domains. The anticipated relationships between the mutated HER2 domain and tumour type were observed, with extracellular domain mutations predominant in bladder cancer, kinase domain missense mutations in breast and colon cancer, and kinase domain insertions in lung cancer. Missense mutations were the most common class of genomic alteration (74%), followed by in-frame insertions (22%), the latter exclusively affecting the kinase domain. Two tumours contained HER2 insertions/deletions and one an in-frame kinase domain-retaining fusion (GRB7-ERBB2)12,13. HER3 mutations were all missense variants and clustered in the extracellular furin-like and receptor domains. In total, 87% (109 out of 125) of HER2 and 75% (12 out of 16) of HER3 mutations were at positions now known to be mutational hotspots. This pattern of HER2 and HER3 mutations was comparable to the spectrum of non-truncating HER2 and HER3 mutations observed in previously published genomic landscape studies, including The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC)14, although HER2 V777L and Y772_A775dup were more common in our study cohort (13.6% versus 5.3% and 12.0% versus 2.7%, respectively; Extended Data Fig. 3).

Treatment outcomes

When stratified by tumour type, we observed responses to neratinib in patients with HER2-mutant breast, non-small-cell lung, cervical, biliary and salivary cancers, which led to expanded enrolment in several of these tumour types (Fig. 1a, Extended Data Table 1). Neratinib exhibited the greatest degree of activity in patients with breast cancer (n = 25 total, objective response rate at week 8 (ORR8) 32%, 95% confidence interval 15–54%), with responses observed in patients with missense mutations involving the extracellular and kinase domains, as well as insertions in the kinase domain. All patients with breast cancer were classified as HER2-negative (non-amplified) at the time of enrolment as per established guidelines14. Responses were observed in both oestrogen receptor-positive (30%, 6 out of 20) and -negative (40%, 2 out of 5) tumours. Overall, these breast cancer data are generally consistent with a previous report15. In patients with lung cancer (n = 26), in which insertions in exon 20 predominate, we observed only one objective response. Of note, HER2 exon 20 insertions are paralogous of EGFR exon 20 insertions, which are resistant to first- and second-generation EGFR tyrosine kinase inhibitors16. Notably, the only patient with lung cancer to achieve a response evaluation criteria in solid tumours (RECIST) response had a kinase domain missense mutation (L755S). Despite the low response rate, the median progression-free survival in recurrent lung cancer was 5.5 months, with 6 patients remaining on therapy for more than 1 year, which compares favourably to second-line chemotherapy and immune checkpoint inhibitors17, suggesting that neratinib may have a positive effect on the natural history of this disease. Responses were also observed in biliary and cervical cancers, and enrolment is ongoing in these cohorts to define this activity better. No responses were observed in bladder cancer (n = 16) or colorectal cancer (n = 12), suggesting lineage-dependent resistance to single-agent pan-HER kinase inhibition in these tumour types. In summary, among the HER2-mutant cohorts, breast cancer met the primary endpoint for efficacy, whereas lung, colorectal and bladder cancers did not. For the remaining tumour-specific cohorts, enrolment is continuing and they have therefore not undergone final efficacy analysis. Despite preclinical data to suggest that HER3 mutations can be oncogenic drivers, no responses to neratinib were observed in patients with HER3-mutant tumours.

When stratified by mutant allele, responses were observed in patients with tumours containing HER2 S310, L755, V777L, G788_P780dup and Y772_A775dup mutations (Fig. 1b). Among patients with HER2 kinase domain hotspot missense mutations (n = 42), responses were noted in four unique tumour types (breast, biliary, lung and salivary gland). By allele, we observed responses in several kinase domain mutants including L755S (n = 4), V777L (n = 4) and L869R (n = 1). In patients with HER2 hotspot extracellular domain mutations (S310, n = 30), responses were observed in breast, cervical and biliary cancers (n = 1 for each), but not in bladder cancer, the cancer type in which these mutations predominate. Similarly, in patients with HER2 exon 20 insertions (n = 28), responses were observed in two patients with breast cancer, but none were seen in patients with lung cancer, in which this class of alteration is most common. In exon 20 insertions, preservation of glycine at the 770 position, which seems to facilitate binding of covalent HER kinase inhibitors such as neratinib, did not predict for response as previously suggested by preclinical modelling18 (Extended Data Fig. 4). Similarly, the number of amino acids involved in the insertion did not seem to predict outcome, with responses observed in patients with both 3 (G788_P780dup) and 4 (Y772_A775dup) amino acid insertions. Finally, among the 15 patients with HER2 mutations not known to be hotspots, only one responded to neratinib. Notably, this response occurred in a patient with breast cancer and a complex insertion/substitution (L755_E757delInsS), which, to our knowledge, has not been observed previously. Although this case illustrates that the tumours of some patients may be addicted to truly private oncogenic drivers (those arising in only a single patient), it is also noteworthy that this insertion occurs in a domain that is the target of recurrent insertions. The absence of clinical activity in the remaining 14 patients with cancers with non-hotspot mutations suggests that, although the recurrence of a mutation in HER2 is insufficient to define it as sensitizing to a HER2 kinase inhibitor, the absence of recurrence (that is, mutations that do not occur at hotspot positions) provides circumstantial evidence that the alteration is unlikely to be a driver.

Although the overall numbers of patients in each subgroup preclude formal statistical comparison, integrating efficacy, mutational and lineage data, we observed that clinical benefit from neratinib therapy appeared to vary as a function of both mutational and disease context.
(Fig. 2). In tumour types sensitive to neratinib therapy, such as breast, biliary and cervical cancers, responses were collectively observed across all types and classes of HER2 mutations. By contrast, in lung cancer, a tumour type that exhibits modest sensitivity to neratinib, response was limited to a patient with a HER2 kinase domain missense mutation—a class of mutation with greater in vitro sensitivity to neratinib5. Finally, in tumour types with intrinsic lineage-based resistance to neratinib, such as bladder and colorectal cancers, responses were not observed regardless of the HER2 mutation, type or class.

**Safety**

All patients received neratinib with mandatory anti-diarrhoeal prophylaxis. With this regimen, the rate of grade 3 diarrhea was 22% (Extended Data Table 2), consistent with previous experience19. Among patients who developed grade 3 diarrhea, the median time to onset was 10 days and the median duration of the diarrhea episode was 2 days. Patients were typically managed with dose interruption and reduction, with only 2.8% permanently discontinuing therapy owing to diarrhea. The remainder of adverse events were predominantly low-grade.

Central confirmation of HER2 and HER3 mutations

There is active debate within the cancer research community as to whether central confirmation of mutational status before study entry is optimal for determining trial eligibility for precision medicine studies. To define the reproducibility of local mutational testing, DNA from archival formalin-fixed paraffin-embedded tumour and plasma samples were re-sequenced (see Methods). A total of 33 patients (26 HER2-mutant, 7 HER3-mutant) were excluded from this concordance analysis because the local test used was the same as the central tumour assay being evaluated. Of the remaining 99 patients with HER2 mutations, adequate material for tumour genomic testing was obtainable for 26 patients. Overall, concordance in the remaining patients based on central tumour and/or plasma sequencing was 95% (69 out of 73), with 38 patients assessed by tissue and plasma, 14 by tissue alone, and 21 by plasma alone. Central testing identified one locally reported mutation (V773M) as a germline polymorphism and this patient, with renal cell carcinoma, had progressive disease at first scan. Central testing in the four cases in which the HER2 mutation could not be confirmed passed all quality-control metrics, but in two patients the tissue used for local testing, raising the possibility that tumour heterogeneity was involved in the discordance. None of the patients with discordant HER2 results responded to neratinib, and their median progression-free survival was only 43 days (range: 5–58 days). Among the 9 patients eligible for concordance testing with HER3 mutations, tumour tissue was available for central sequencing in 8 patients, and overall concordance was 75% (6 out of 8).

Genomic modifiers of response

Given the variability of treatment response, even among patients with the same tumour lineage and HER2-mutant allele, we sought to identify other genomic modifiers of response through broader genomic characterization of tumour-derived DNA (see Methods). First, we...
explored the relationship between ERBB2 amplification and outcome, as this is a well-established predictor of response to HER2-targeted therapies in patients lacking HER2 mutations. In total, 17% of patients (15 out of 86) had concurrent HER2 mutations and gene amplification. Amplifications preferentially targeted the mutant allele locus (86%, 12 out of 14 evaluable). Using a dichotomous definition of clinical benefit (stable disease or partial response lasting at least 24 weeks), ERBB2 amplification did not correlate with outcome ($P = 0.50$; Fig. 3), suggesting that in the presence of HER2 mutations, amplification may not confer additional sensitivity to irreversible HER kinase inhibitors. We also explored the relationship of ERBB2 mutation clonality on outcomes. In the 74 patients with adequate material to allow definitive assessment of ERBB2 mutant clonality, the mutation was clonal in 95% (70 out of 74; Extended Data Fig. 5a). None of four patients with a subclonal ERBB2 mutation achieved clinical benefit.

Hypothesizing that tumours with an increased tumour mutational burden (TMB) might be more likely to acquire HER2 mutations without developing oncogenic dependence (that is, passenger mutations), we evaluated whether overall TMB status affected outcome. Using a previously validated cut-off ($\geq 13.8$ non-synonymous mutations per megabase of DNA$^2$), 20% of patients (17 out of 86) met criteria for a high TMB. In total, 24% of patients (16 out of 66) without clinical benefit versus 5% of patients (1 out of 20) with benefit met criteria for a high TMB, a trend that did not reach statistical significance ($P = 0.10$).

Next, we evaluated whether the pattern of co-mutations affected clinical benefit in the subset of patients where broader profiling was available ($n = 86$). In patients with HER2-mutant disease, coincident mutations in TP53 and HER3 were enriched in patients with no clinical benefit (nominal $P = 0.018$ and $P = 0.064$, respectively; Fig. 3). Although not significant after correcting for multiple hypothesis testing, potentially owing to the relatively small sample size, it is noteworthy that no patients with clinical benefit possessed co-mutation of HER2 and HER3. Concurrent mutation of these genes was observed in multiple cancer types (breast $n = 3$, bladder $n = 2$, gastroesophageal $n = 2$, colorectal $n = 1$ and pancreatic $n = 1$) and involved a variety of unique HER2 and HER3 mutations ($n = 8$ and $n = 9$, respectively). Expanding our analysis to genomic activation at the pathway level, we identified somatic mutations of known oncogenic potential and grouped them by those involving the receptor tyrosine kinase (RTK)/RAS/RAF and PIK3CA/AKT/mTOR pathways, and cell cycle checkpoints (Extended Data Fig. 5b). In this analysis, concurrent aberrations in cell cycle checkpoints were associated with lack of clinical benefit ($P = 0.043$), and activation of RTK/RAS/RAF also trended towards a worse outcome ($P = 0.060$). The association between the cell-cycle pathway and lack of clinical benefit seems to be primarily driven by TP53 mutations, losing significance upon removal of TP53 mutations ($P = 0.769$). Interestingly, activation of the PI3K/AKT/mTOR pathway, an established negative predictor of response to HER2-targeted therapy in HER2-amplified breast cancer$^{20-22}$, did not adversely affect the likelihood of clinical benefit ($P = 0.753$). It is possible that the clinical impact of concurrent gene/pathway activation may vary by tumour type, and future disease-specific studies are needed to define these associations better. Although these were exploratory analyses that will require confirmation, our results suggest that concurrent activation of specific genes as well as pathways may act as an additional modifier of response beyond cancer type and specific HER2 mutant allele.

**Discussion**

The ability to profile cancer comprehensively at the point of care has made possible the opportunity to personalize therapy for each patient based on the compendium of genomic alterations identified$^{23}$. Despite the promise of this approach, implementing this paradigm in clinical practice has been hampered by considerable gaps in knowledge about the biological and clinical importance of most genomic variants identified$^{24}$. This challenge is exemplified by the marked diversity and wide distribution of HER2 and HER3 mutations in human cancers,
as well as by the difficulty of generating preclinical models of these mutations that correctly recreate their biology in patients. To our knowledge, SUMMIT provides the first comprehensive dataset on the clinical actionability of HER2 and HER3 mutations. We found that HER2 mutations are associated with HER2-dependence in a subset of patients with HER2-mutant tumours, but that response to HER kinase inhibition varies a function of the individual mutant variant, the tumour type as well as the pattern of co-mutations present.

Although we identified promising preliminary activity for neratinib in breast, biliary and cervical cancers, the response rate in these tumours was still lower than with approved therapies that target oncogenic alterations in EGFR, ALK, ROS1 and BRAF. The low response rate in lung cancer, in which HER2 mutations exhibit mutually exclusivity with other known drivers, is also notable and may in part reflect a lower potency of neratinib inhibition in Y772_A775dup compared to other insertions or missense mutants. Successfully targeting HER2 activation in other contexts has historically necessitated drug combinations. For example, single-agent trastuzumab has a response rate of only approximately 20% in ERBB2-amplified breast cancer. By contrast, the overall survival in ERBB2-amplified breast and gastrointestinal cancers is markedly improved by adding trastuzumab to chemotherapy. More recently, the intensification of HER2 inhibition through the combination of two HER2-targeted agents has been shown to result in synergistic efficacy in patients with ERBB2-amplified breast or colorectal cancers, as well as in HER2-mutant colorectal cancer xenografts. Cumulatively, these data suggest that combining neratinib with another HER2-targeted therapy is a rational next step, as well as by the difficulty of generating preclinical models of these mutations that correctly recreate their biology in patients. To our knowledge, SUMMIT provides the first comprehensive dataset on the clinical actionability of HER2 and HER3 mutations. We found that HER2 mutations are associated with HER2-dependence in a subset of patients with HER2-mutant tumours, but that response to HER kinase inhibition varies a function of the individual mutant variant, the tumour type as well as the pattern of co-mutations present.

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SUMMIT provides additional insights into the conduct of molecularly driven oncology studies. Our ability to understand the complex interactions between tumour lineage, individual HER2 variant and response to neratinib was only possible because of the relatively large size of this study (n = 141). By comparison, many of the ‘master umbrella’ protocols that are currently underway are designed to enroll a maximum of 30–40 patients into each genomically defined treatment arm. Our experience suggests that many studies of this size may be inadequately powered to identify the subgroups with true efficacy, assuming that most genomic alterations will not predict for tumour-type agnostic efficacy. SUMMIT also demonstrates the feasibility of enrolling patients based on local testing, with patients treated on the basis of 30 unique sequencing assays performed in 25 different laboratories. Despite this, concordance on retrospective central review was extremely high (96%).

An important impediment to progress in oncology has been the limited availability of preclinical model systems that accurately recreate the complex biology of human cancer. Although important strides have been made, the wide-scale profiling of cancer in the clinic provides the potentially transformative opportunity to interrogate cancer biology at the bedside in a manner previously only possible at the bench. Here,

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we demonstrate how this opportunity can be leveraged to probe the biology of a diverse set of HER2 and HER3 mutations across a variety of solid tumours through pharmacological HER kinase inhibition in patients. In doing so, we found that response to pharmacological inhibition was based on the characteristics of both tumour type and genomic variant to a degree that was not predicted by established preclinical models. In summary, SUMMIT demonstrates how the clinical trial can become an important tool in refining our understanding of the biological dependencies in human cancers.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to methods, along with any additional Extended Data display items and Online Content.

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METHODS

Patients. Eligible patients had histologically confirmed advanced solid tumours harbouring HER2 or HER3 mutations, an Eastern Cooperative Oncology Group (ECOG) performance score of 0–2 and an unlimited number of previous therapies. Patients with previous exposure to HER kinase inhibitors and unstable brain metastases were excluded. HER2 and HER3 mutations were determined by local tumour testing as routinely performed or ordered by each participating site. In total, 85% (120 out of 141) of enrolled patients were identified by next-generation sequencing (NGS) tests. In 5% of cases (97 out of 120), the next-generation sequencing assay included full exon coverage for ERBB2 or ERBB3, whereas in 19% (23 out of 120) of cases, only select exons or hotspots were included in the assay design. The remaining 15% (21 out of 141) of patients were enrolled via RT–PCR, Sanger, pyrosequencing, or mass spectrometry-based sequencing methods. The study was approved by the institutional review board or independent ethics committee at each site and complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws. Written informed consent was obtained from all participants.

Study design, treatment and endpoints. This was a multi-cohort basket study of patients with solid tumours harbouring HER2 and HER3 mutations. Patients with HER2-mutant tumours were enrolled into one cohort. Patients known to contain both HER2 and HER3 mutations at the time of enrolment were assigned to the HER2-mutant cohort. Patients were treated with neratinib 240 mg daily on a continuous basis with mandatory lopremda prophylaxis during cycle 1. The primary endpoint was ORR, as assessed by investigators according to RECIST (version 1.1). Secondary endpoints included overall response; progression-free survival, overall survival and safety. Patients who were not evaluable by RECIST were permitted entry and were evaluated for response by 18F-fluorodeoxyglucose-positron emission tomography according to a modified version of the original PET Response Criteria in Solid Tumours (PERCIST; version 1.0)48, referred to here as PET Response Criteria (PRC, Extended Data Table 3).

Assessments. Disease assessments with computed tomography, magnetic resonance imaging or combined positron emission tomography–computed tomography (for those evaluated by PRC) were performed at baseline and then every 8 weeks until disease progression, death or withdrawal. Adverse events were graded by the investigator according to the Common Terminology Criteria for Adverse Events (version 4.0) until day 28 after discontinuation of study treatment. Genomic biomarker studies. All samples were assigned anonymized identifiers by the study sponsor based on the order of study enrolment. Both tumour DNA and tumour-derived cell-free DNA in plasma were collected with the goals of confirming locally reported HER2/3 mutations as well as evaluating how ERBB2 and ERBB3 copy number and clonality as well as co-mutational pattern affected outcome. Collection of archival tumour and plasma samples was mandatory for all patients. Next-generation sequencing was performed using targeted sequencing of pretreatment DNA from formalin-fixed paraffin-embedded tumour and matched blood specimens (preferentially) and cell-free DNA (if tumour was not available or was inadequate). A custom hybridization-based exon capture next-generation sequencing test was also performed on pretreatment cell-free DNA in a subset of patients with HER2-mutant disease.

Central sequencing confirmation. For patients with adequate material, DNA from formalin-fixed paraffin-embedded (n = 91) or tumour-derived cell-free DNA from plasma (n = 15) and matched germline DNA (n = 102) underwent targeted next-generation sequencing assay using Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT)2, producing an average of 738-fold coverage per tumour (range: 253–1,383). In brief, this assay uses a hybridization-based exon capture designed to capture all protein-coding exons and select introns of oncoproteins, tumour-suppressor genes and key members of pathways that may be druggable through targeted therapies. This assay allows mutation testing of up to 431 genes in 741 patients (n = 88) key cancer-associated genes were analysed (Supplementary Information). Sequencing data were analysed as previously described to identify somatic single-nucleotide variants, small insertions and deletions, copy number alterations and structural arrangements49. In addition, hotspot alterations were identified using an adaptation of a previously described method4 applied to a cohort of 24,592 sequenced human cancers50. For gene-level analysis, select genes within our targeted 341–410 MSK-IMPACT panel related in the RTK/RAS/RAF, PI3KCA/AKT/ mTOR, and cell cycle checkpoint pathways were selected using the KEGG pathway database41. For gene-level analysis, only potentially oncogenic alterations in the selected genes were included and determined to be actionable using the Oncomine Genomics Oncogenic Signature Set (www.oncomine.org). A curatated knowledge base of the oncoenic effects and treatment implications of mutations and cancer genes (http://www.oncokb.org/) was used to estimate tumour purity and ploidy, and total and allele-specific copy number42. Tumour samples with purity less than 20% were excluded from this analysis. Focal HER2 amplifications for tumours with MSK-IMPACT and FACETS data were inferred using the following criteria: fold change ≥ 1.5 (MSK-IMPACT tumour-normal sequencing coverage ratio) and total HER2 copy number ≥ 4 copies (FACETS-derived total copy number). To infer clonality of each HER2 mutation, cancer cell fractions were estimated with 95% confidence intervals by integrating FACETS-derived joint segmentation and MSK-IMPACT mutation data as input into the ABSOLUTE algorithm44 (version 1.0.6). Mutations were identified in the following order of the criteria: clonal if the estimated cancer cell fractions > 0.85, otherwise subclonal. For patients with HER2 amplification, the mutation copy number (mutation multiplicity) was calculated as previously described43 to infer amplification of the mutant allele when the mutation multiplicity was greater than half of the total HER2 copy number.

TMB and MSI. TMB, defined as the number of non-synonymous mutations per megabase, was calculated for patients with MSK-IMPACT sequencing data (n = 106)46. MSI was assessed for patients with HER2-mutant tumours with matched germline DNA sequencing data (n = 89) using an orthogonal bioinformatics tool, MSISensor44. Furthermore, mutations were decomposed into the 30 constituent mutational signatures as described previously52. In brief, MSISensor scores < 10 were classified as microsatellite stable and > 10 were considered MSI-high using a previously validated cut-off score46. Those with a MSISensor score of < 10 but having evidence of a dominant mismatch repair mutational signature were also considered MSI43,47.

Statistical analysis. For each HER2-mutant tumour type and the HER3-mutant cohort, a Simon optimal two-stage design with a true ORR ≤ 10% was considered unacceptable (null hypothesis), while a true ORR ≥ 30% (alternative hypothesis) was considered of further study. Efficacy in each cohort was analysed independently and the study was not designed to compare efficacy across cohorts formally. All patients who received at least one dose of neratinib were included in the safety and efficacy analyses. All data reflect an interim data-cut taken on 10 March 2017 from patients enrolled up to 16 December 2016 (Extended Data Fig. 6). Most patients were off therapy at the time of data analysis (Extended Data Table 4). Progression-free survival was estimated using the Kaplan–Meier method. The study is registered at http://www.clinicaltrials.gov, under the identifier NCT01953296. Individual associations among genomic changes and response were assessed by either Fisher’s exact or chi-squared tests (where appropriate) and corrected for multiple hypothesis testing using Benjamini–Hochberg correction. Chi-squared or Fisher’s exact tests were performed to compare gene-level and pathway-level associations between the dichotomous clinical benefit groups. P values were corrected for multiple hypothesis testing using Benjamini–Hochberg correction. HER2 and HER3 lollipop distribution plots were generated using ProteinPaint49. All other figures were generated using R software (http://www.R-project.org/).

This clinical trial was not randomized and investigators were not blinded to treatment allocation and outcome assessment.

Data availability. All datasets generated during and/or analysed during the current study, including patient-level clinical data as well as all sequencing data have been deposited and are publically available in the eBioPortal for Cancer Genomics under the accession code SUMMIT, Nature, 2018 (http://www.bioproject.org/study?sid= summit_2018).

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Extended Data Figure 1 | Design of SUMMIT study. Five tumour-specific HER2 (ERBB2)-mutant cohorts were pre-specified (endometrial, gastroesophageal, ovarian, colorectal and bladder/urinary tract). In addition, a sixth ‘solid tumour (not otherwise specified, NOS)’ HER2-mutant cohort allowed for the enrolment of patients with any other cancer types. A sufficient number of patients with breast, cervical, biliary and lung cancer were enrolled in the solid tumours (NOS) cohort to permit independent efficacy analysis using the same design as the pre-specified cohorts. Patients with HER3 (ERBB3)-mutant tumours were enrolled in a HER3-specific cohort regardless of tumour type. CBR, clinical benefit rate; cfDNA, cell-free (tumour) DNA; CI, confidence interval.
Extended Data Figure 2 | Distribution of HER2 and HER3 mutations positioned by their amino acid coordinates across the respective protein domains. a, b, HER2 (a) and HER3 (b) mutations (125 and 16 mutations, respectively). Each unique mutation is represented by a circle, with the circle size and number representing the frequency, and coloured to show the mutation class as indicated in the legend. The corresponding amino acid change and common hotspot mutations (shown in pink) are labelled next to the circles.
Extended Data Figure 3 | Spectrum of HER2 and HER3 mutations observed in the neratinib study versus TCGA, ICGC and other public datasets. a, b, Distribution of HER2 (a) and HER3 (b) mutations observed across our cohort in comparison to the spectrum of HER2 and HER3 mutations (reflected lollipop) from publically available datasets (TCGA, ICGC and other published studies).
Extended Data Figure 4 | Distribution and outcome of 28 HER2 exon 20 insertions. **a**, Percentage best change and PFS plots corresponding to each type of exon 20 insertion (colour coded by synonymous amino acid change). Three cases with no change are indicated in colour-coded circles above the x axis. **b**, Zoomed-in schematic of all exon 20 insertions positioned by their amino acid coordinates and frequencies. **c**, Five unique types of exon 20 insertions observed in the study with the resulting full amino acid sequences (insertion indicated in red).
Extended Data Figure 5 | Genomic modifiers of response and outcome by treatment duration. a, Cancer cell fractions with 95% confidence intervals and clonality status of all HER2 mutations in 74 patients with sufficient sequencing data ordered by increasing clinical benefit (weeks on therapy). b, Comparison of the percentage activation of known oncogenic alterations in the three pathways between the patients of clinical benefit (n = 20, biologically independent samples) and no benefit (n = 66, biologically independent samples). Nominal Fisher’s P values are shown.
Assessed for eligibility (n=175)

Excluded (n=32)
- Not meeting eligibility criteria (n=31)
- Unknown (n=1)

Enrolled (n=143)

Received neratinib (n=141)

Analyzed (n=141)

Discontinued neratinib (n=131)
- Disease progression (n=104)
- Clinical deterioration (n=8)
- Adverse event (n=5)
- Investigator request (n=5)
- Withdrawal of consent (n=5)
- Death (n=3)
- Lost to follow-up (n=1)

Extended Data Figure 6 | SUMMIT CONSORT diagram.
## Extended Data Table 1 | Patient demographics and efficacy by cohort

| Characteristic                  | HER2 (n=25) | Lung (n=26) | Bladder (n=16) | Colorectal (n=12) | Biliary tract (n=9) | Cervical (n=5) | Endometrial (n=7) | Gastric- esophageal (n=5) | Ovarian (n=4) | NOS (n=16) | NOS (n=16) |
|--------------------------------|-------------|-------------|----------------|-------------------|--------------------|---------------|-------------------|--------------------------|---------------|-----------|-----------|
| **Median (range), years**      |             |             |                |                   |                    |               |                   |                          |               |           |           |
| <65 years, n (%)               | 7 (42.3)    | 6 (37.5)    | 6 (37.5)       | 5 (41.7)          | 2 (22.2)           | 1 (20.0)      | 2 (20.0)          | 0 (0)                    | 0 (0)         | 1 (6.3)   |           |
| 65 years, n (%)                | 17 (68.0)   | 14 (53.8)   | 10 (62.5)      | 7 (58.3)          | 6 (66.7)           | 4 (90.0)      | 5 (100)           | 5 (100)                  | 4 (100)       | 11 (68.8) | 12 (70.0) |
| **ECOG PS, n (%)**             |             |             |                |                   |                    |               |                   |                          |               |           |           |
| 0                              | 0 (0)       | 1 (3.8)     | 1 (6.3)        | 0 (0)             | 1 (11.1)           | 0 (0)         | 0 (0)             | 0 (0)                    | 0 (0)         | 1 (6.3)   | 0 (0)     |
| 1                              | 3 (12.0)    | 2 (12.5)    | 2 (12.5)       | 4 (33.3)          | 3 (33.3)           | 1 (43.3)      | 3 (33.3)          | 2 (20.0)                 | 2 (20.0)      | 6 (37.5)  | 1 (6.3)   |
| 2                              | 1 (4.0)     | 1 (3.8)     | 0 (0)          | 0 (0)             | 1 (11.1)           | 0 (0)         | 0 (0)             | 0 (0)                    | 0 (0)         | 2 (12.5)  | 11 (68.8) |
| 3                              | 20 (80.0)   | 7 (26.9)    | 4 (25.0)       | 5 (41.7)          | 3 (33.3)           | 2 (40.0)      | 4 (57.1)          | 2 (40.0)                 | 4 (100)       | 7 (43.8)  | 4 (25.0)  |
| **Prior systemic lines, n (%)**|             |             |                |                   |                    |               |                   |                          |               |           |           |
| None                           | 3 (12.0)    | 2 (12.5)    | 2 (12.5)       | 4 (33.3)          | 3 (33.3)           | 1 (43.3)      | 3 (33.3)          | 2 (20.0)                 | 2 (20.0)      | 6 (37.5)  | 1 (6.3)   |
| 1                              | 2 (8.0)     | 6 (23.1)    | 9 (56.3)       | 3 (26.0)          | 2 (22.2)           | 3 (90.0)      | 2 (20.0)          | 1 (20.0)                 | 0 (0)         | 2 (12.5)  | 11 (68.8) |
| 2                              | 20 (80.0)   | 7 (26.9)    | 4 (25.0)       | 5 (41.7)          | 3 (33.3)           | 2 (40.0)      | 4 (57.1)          | 2 (40.0)                 | 4 (100)       | 7 (43.8)  | 4 (25.0)  |
| Median time from metastasis to aorment, years (range) | 2.94 (0.1-15.0) | 0.03 (0.1-3.1) | 0.09 (0.2-2.9) | 1.14 (0.0-2.7) | 1.00 (0.0-2.8) | 1.40 (0.0-3.4) | 0.43 (0.0-2.4) | 0.90 (0.0-4.4) | 7.54 (1.1-17.7) | 1.36 (0.0-9.4) | 1.13 (0.3-4.8) |
| **Outcome**                    |             |             |                |                   |                    |               |                   |                          |               |           |           |
| **ORR at week 8, n (%)**       |             |             |                |                   |                    |               |                   |                          |               |           |           |
| (95% CI)                       | 8 (32.0)    | 1 (3.8)     | 0 (0)          | 0 (0)             | 2 (22.2)           | 1 (20.0)      | 0 (0)             | 0 (0)                    | 0 (0)         | 1 (6.3)   | 0 (0)     |
|                               | [14.8-53.3] | [0.1-10.6]  | [0.0-20.6]     | [0.0-20.6]        | [2.8-40.0]         | [1.5-71.6]    | [0.0-41.6]        | [0.0-41.6]               | [0.0-40.2]    | [0.2-30.2] | [0.0-20.6] |
| **ORR, n (%)**                 |             |             |                |                   |                    |               |                   |                          |               |           |           |
| (95% CI)                       | 6 (24.0)    | 1 (3.8)     | 0 (0)          | 0 (0)             | 0 (0)              | 0 (0)         | 0 (0)             | 0 (0)                    | 0 (0)         | 0 (0)     | 0 (0)     |
|                               | [0.4-49.1]  | [0.1-19.9]  | [0.0-20.6]     | [0.0-20.6]        | [0.0-20.6]         | [0.0-20.6]    | [0.0-30.6]        | [0.0-20.6]               | [0.0-20.6]    | [0.0-20.6] | [0.0-20.6] |
| **Clinical benefit rate, n (%)**|             |             |                |                   |                    |               |                   |                          |               |           |           |
| (95% CI)                       | 10 (40.0)   | 11 (42.3)   | 2 (18.8)       | 1 (3.8)           | 3 (33.3)           | 3 (60.0)      | 2 (20.0)          | 1 (20.0)                 | 0 (0)         | 2 (12.5)  | 2 (12.5)  |
|                               | [21.1-61.3] | [23.4-63.1] | [4.0-49.6]     | [7.9-70.1]        | [14.1-74.7]        | [7.8-71.6]    | [3.7-71.6]        | [0.0-90.2]               | [45.6]        | [1.9-38.3] |
| **Median PFS, months**         | 3.5         | 5.5         | 1.8            | 1.8               | 2.8               | 20.1          | 2.6               | 1.7                      | 2.1           | 1.9       | 1.7       |
## Extended Data Table 2 | Treatment-emergent adverse events (occurring in ≥ 10% of patients)

| Adverse event, n (%) | Neratinib monotherapy (N=141) | Any grade | Grade ≥3 |
|----------------------|--------------------------------|-----------|----------|
| Diarrhoea            | 104 (73.8)                     | 31 (22.0)*|          |
| Nausea               | 61 (43.3)                      | 3 (2.1)   |          |
| Vomiting             | 58 (41.1)                      | 3 (2.1)   |          |
| Constipation         | 49 (34.8)                      | 2 (1.4)   |          |
| Fatigue              | 45 (31.9)                      | 5 (3.5)   |          |
| Decreased appetite   | 40 (28.4)                      | 1 (0.7)   |          |
| Abdominal pain       | 33 (23.4)                      | 7 (5.0)   |          |
| Anaemia              | 22 (15.6)                      | 10 (7.1)  |          |
| Dyspnoea             | 18 (12.8)                      | 5 (3.5)   |          |
| Dehydration          | 17 (12.1)                      | 8 (5.7)   |          |
| Aspartate aminotransferase increased | 15 (10.6) | 5 (3.5) |          |
| Asthenia             | 15 (10.6)                      | 1 (0.7)   |          |
| Weight decreased     | 15 (10.6)                      | 0         |          |

### Characteristics of diarrhoea

| Action taken with neratinib, n (%) | Neratinib monotherapy (N=141) | Any grade | Grade ≥3 |
|-----------------------------------|--------------------------------|-----------|----------|
| Permanent discontinuation         | 4 (2.8)                        |           |          |

**Serious** diarrhoea, n (%): 15 (10.6)

**Median (range) number of grade 3 diarrhoea episodes per patient**: 1 (1–12)

**Median (range) duration of grade 3 diarrhoea episode, days**: 2 (1–8)

**Median (range) time to first grade 3 diarrhoea episode, days**: 10 (4–87)

*All events of grade 3.
†Serious adverse event as defined per study protocol.
**Extended Data Table 3 | PET response criteria**

| Response category       | Based on sum of SUV\(_{\text{max}}\) from 1 to 5 target lesions. Each target lesion with initial SUV\(_{\text{max}}\) of >1.5 × normal liver background SUV\(_{\text{max}}\). |
|-------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Complete metabolic (CMR)| • Reduction of SUV\(_{\text{max}}\) of all target lesions to less than normal liver background SUV\(_{\text{max}}\) (for non-brain lesions) or less than normal brain background SUV\(_{\text{max}}\) (for brain lesions)  |
|                         | • The reduction of all other FDG-avid lesions consistent with disease to less than normal liver background SUV\(_{\text{max}}\) |
| Partial metabolic (PMR) | • Sum of SUV\(_{\text{max}}\) of all target lesions is decreased by ≥30% compared to baseline sum of SUV\(_{\text{max}}\) of all target lesions  |
|                         | • No new lesions                                                                                                                     |
| Stable metabolic disease (SMD) | Not satisfying the criteria for CMR, PMR, PMD, or NE                                                                                     |
| Progressive metabolic disease (PMD) | • Sum of SUV\(_{\text{max}}\) of all target lesions is increased by ≥30%  |
|                         | • Appearance of one or more unequivocal new FDG-avid lesions                                                                         |
| Not evaluable (NE)      | • Missing FDG-PET series or incomplete anatomy at follow-up timepoint  |
|                         | • A PET/CT scanner change from baseline                                                                                              |
|                         | • Variation in FDG uptake time ≥15 minutes compared to baseline                                                                         |
|                         | • Change in reconstruction algorithm                                                                                                   |

CT, computed tomography; FDG-PET, \(^{18}\)F-fluorodeoxyglucose positron-emission tomography; SUV\(_{\text{max}}\), maximum standardized uptake value.
Extended Data Table 4 | Patient disposition by cohort

| Characteristic                          | HER2     | HER3     |
|----------------------------------------|----------|----------|
| Patients continuing on treatment, n (%)| 1 (4)    | 1 (6.2)  |
| Treatment discontinuation, n (%)       | 2 (10.0)| 15 (93.6)|
| Death                                  | 0 (0)    | 0 (0)    |
| Disease progression                    | 22 (91.3)| 12 (75.0)|
| Clinical deterioration                 | 0 (0)    | 3 (11.1)|
| Adverse Event                          | 0 (0)    | 3 (11.1)|
| Investigator Request                   | 0 (0)    | 0 (0)    |
| Withdrawal of consent                  | 2 (8.0)  | 0 (0)    |
| Lost to follow-up                      | 0 (0)    | 1 (4.3)  |
| Subjects ended study, n (%)            | 15 (60.0)| 14 (87.5)|
| Death                                  | 12 (48.0)| 13 (81.3)|
| Withdrawal of consent                  | 2 (8.0)  | 0 (0)    |
| Lost to follow-up                      | 1 (4.0)  | 1 (6.3)  |
| Other                                  | 0 (0)    | 0 (0)    |

NOS, not otherwise specified.
Experimental design

1. Sample size
   Describe how sample size was determined.
   
   The sample size required depends on the objective response rates at 8 weeks (ORR8). For each cohort, using Simon’s optimal 2-stage design (with significance level 10% and power of 80%), a true ORR8 of 10% or less will be considered unacceptable (null hypothesis) whereas a true ORR8 of minimally 30% (alternative hypothesis) will merit further study. In the first stage, enrollment will continue until 7 patients per the Simon 2-stage optimal design. If no responses are observed, enrollment in the second stage for the cohort may be discontinued. Otherwise, the second stage will open and 11 additional response evaluable patients will be assessed for a total of 18 patients in the cohort. The null hypothesis will be rejected (for each cohort separately) if at least 4 responses are observed in each cohort. Once the Simon 2-stage criteria are met for the ERBB2 mutant breast cohort, enrollment into this cohort may continue until approximately 50 patients.

2. Data exclusions
   Describe any data exclusions.
   
   The clinical data presented represent an intention to treat population with all patients who received at least one dose of neratinib included in analysis. The genomic data presented include all samples that underwent sequencing and passed routine QA/QC procedures as described and referenced.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   
   As this is a clinical trial, no replication was possible or performed.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   
   There was no randomization.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   
   This was an open label study with no blinding.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters
For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ✓ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ✓ | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ✓ | A statement indicating how many times each experiment was replicated |
| ✓ | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| ✓ | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ✓ | The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
| ✓ | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
| ✓ | Clearly defined error bars |

See the web collection on statistics for biologists for further resources and guidance.

7. Software
Policy information about availability of computer code

SAS EG5.1 by SAS Institute Inc., ABSOLUTE v. 1.0.6, FACETS v. 0.3.9, R v. 3.3.1, MSIsensor v. 0.2

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials and reagents
Policy information about availability of materials

8. Materials availability

No unique materials were used

9. Antibodies

No antibodies were used

10. Eukaryotic cell lines

No eukaryotic cell lines were used

10a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used

10b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used

10c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used

10d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used

11. Description of research animals
Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

No animals were used

Provide details on animals and/or animal-derived materials used in the study.
12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

A detailed summary of the demographics of the human research participants are included in Table 1 and Extended Data Table 1. In addition, patient level demographic data are provided in the cBioPortal project associated with this manuscript.
Author Correction: HER kinase inhibition in patients with HER2- and HER3-mutant cancers

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In the interests of transparency, the authors wish to amend the Competing Interests statement of this Article to read as follows (author initials are in boldface): R.E.C., F.X., L.D.E., G.M., C.F., A.S.L., and R.P.B. are employees of Puma Biotechnology. D.M.H. reports, regardless of relevance, receipt of personal fees from Afara Biotherapeutics, Chugai Pharma, CytomX Therapeutics, Boehringer Ingelheim and AstraZeneca, and research funding from Puma Biotechnology, AstraZeneca and Loxo Oncology. B.T.L. reports research funding from Daiichi-Sankyo. A.D. reports personal fees from Roche. D.B.S. reports receipt of personal fees from Loxo Oncology and Pfizer. J.P.E. is a consultant for BTG International, AstraZeneca and Canon USA. I.A.M. reports receipt of personal fees from Novartis, Genentech Lilly, AstraZeneca, GlaxoSmithKline, Immunomedics, Macrogenics and Seattle Genetics, and research funding to her institution (Vanderbilt-Ingram Cancer Center, Nashville, Tennessee, USA) from Novartis, Genentech and Pfizer. F.M.-B. reports grants from Novartis, AstraZeneca, Calithera, Bayer, Jounce Therapeutics, CytomX, eFFECTOR, Zymeworks, Puma Biotechnology, Curis, Takeda-Millennium, Daiichi-Sankyo, AbbVie, Guardant Health and Takeda, as well as grants and travel-related fees from Taiho, Genentech, Debiopharm Group and Pfizer. F.M.-B. also served as a consultant to Pieris Pharmaceuticals, Dialectica, Sumitomo Dainippon, Samsung Bioepis, Aduro, OrigiMed, Xencor and Jackson Laboratory, and as an advisor to Inflection Biosciences, GRAIL, Darwin Health, Clearlight Diagnostics, Spectrum and Mersana. 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J.B. reports non-financial support and reasonable reimbursement for travel from Roche/Genentech; receipt of fees from and stock ownership in the following companies: Aura Biosciences (including serving on the board of directors 2013–2017), Infinity Pharmaceuticals (including serving on the board of directors 2013–2017), PMV Pharma Biotechnologies (including serving on the scientific advisory board 2014 to the present), Juno Therapeutics (acquired by Celgene) (including serving on the scientific advisory board 2014–2017), GRAIL, (including serving as member or chair of the scientific advisory board 2016–2018), Varian Medical Systems (including serving on the board of directors 2017–2018), Bristol-Myers Squibb (including serving on the board of directors March–September 2018), Seragon (acquired by Roche) (including serving on the scientific advisory board 2013–2014). J.B. also reports stock ownership in ApoGen Biotechnologies (including serving on the scientific advisory board 2014 to the present) and Foghorn Therapeutics (including serving on the board 2017 to the present); serving as co-founder of, receipt of fees from and stock ownership in Tango (formerly Synthetic Lethal) (2016 to the present) and Northern Biologics (formerly Mosaic Biomedicals) (including serving on the scientific advisory board 2013 to the present); receipt of consulting and travel fees from Novartis and Eli Lilly; and serving as co-founder of Venthera. J.B. also reports serving as investigator on a patent licensed to Memorial Sloan Kettering for use of phosphoinositide-3-kinase (PI3K) inhibitors for treatment of vascular malformations and serving as investigator for patents pending assignment to Memorial Sloan Kettering for combination therapy using PDK1 and PI3K inhibitors and inhibition of KMT2D for the treatment of breast cancer. 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