Neratinib + fulvestrant + trastuzumab for HR-positive, HER2-negative, *HER2*-mutant metastatic breast cancer: outcomes and biomarker analysis from the SUMMIT trial

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Abstract

Background: *HER2* mutations are targetable alterations in patients with hormone receptor-positive (HR+) metastatic breast cancer (MBC). In the SUMMIT basket study, patients with *HER2*-mutant MBC received neratinib monotherapy, neratinib + fulvestrant, or neratinib + fulvestrant + trastuzumab (N + F + T). We report results from 71 patients with HR+, *HER2-*

mutant MBC, including 21 (seven in each arm) from a randomized substudy of fulvestrant versus fulvestrant + trastuzumab (F + T) versus N + F + T.

Patients and methods: Patients with HR+ HER2-negative MBC with activating *HER2* mutation(s) and prior cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) therapy received N + F + T (oral neratinib 240 mg/day with loperamide prophylaxis, intramuscular fulvestrant 500 mg on days 1, 15, and 29 of cycle 1 then q4w, intravenous trastuzumab 8 mg/kg then 6 mg/kg q3w) or F + T or fulvestrant alone. Those whose disease progressed on F + T or fulvestrant could cross-over to N + F + T. Efficacy endpoints included investigator-assessed objective response rate (ORR), clinical benefit rate (RECIST v1.1), duration of response, and progression-free survival (PFS). Plasma and/or formalin-fixed paraffin-embedded tissue samples were collected at baseline; plasma was collected during and at end of treatment. Extracted DNA was analyzed by next-generation sequencing.

Results: ORR for 57 N + F + T-treated patients was 39% [95% confidence interval (CI) 26% to 52%); median PFS was 8.3 months (95% CI 6.0–15.1 months). No responses occurred in fulvestrant- or F + T-treated patients; responses in patients crossing over to N + F + T supported the requirement for neratinib in the triplet. Responses were observed in patients with ductal and lobular histology, 1 or   1 *HER2* mutations, and co-occurring *HER3* mutations. Longitudinal circulating tumor DNA sequencing revealed acquisition of additional *HER2* alterations, and mutations in genes including *PIK3CA*, enabling further precision targeting and possible re-response.

Conclusions: The benefit of N + F + T for HR+ *HER2*-mutant MBC after progression on CDK4/6is is clinically meaningful and, based on this study, N + F + T has been included in the National Comprehensive Cancer Network treatment guidelines. SUMMIT has improved our understanding of the translational implications of targeting *HER2* mutations with neratinib-based therapy.

INTRODUCTION

Somatic activating mutations in the *ERBB2 (HER2)* gene in the absence of gene amplification or overexpression are present in ~2% of primary breast cancers,[1–4] in 3% −5% of patients with hormone receptor-positive (HR+) metastatic breast cancer (MBC), and further enriched (5%−8%) in patients with lobular histology.[3,5,6] A subset of *HER2* mutations, including the most common hotspot mutations, have been confirmed to be oncogenic and associated with poor prognosis.[1,5–13] Single-nucleotide variants of *HER2* are predominantly localized to the extracellular, transmembrane, and kinase domains, whereas small insertions are most commonly found in exon 20.[4,9,14] In MBC, *HER2* mutations confer resistance to endocrine therapies due to cross-talk between HER2 and estrogen receptor (ER) signaling pathways.[8,11]

Neratinib is an oral, irreversible, pan-HER tyrosine kinase inhibitor (TKI) with preclinical and clinical activity in patients with *HER2*-mutant tumors.[7,10,12,15–18] Treatment with

neratinib overcomes endocrine resistance in HR+ *HER2*-mutant breast cancer cell lines and xenografts.[8,11] The efficacy of neratinib-based therapy in patients with HR+ *HER2*-mutant MBC was evaluated in the SUMMIT trial (NCT01953926); SUMMIT was hypothesis generating and its basket design enabled evolution of MBC cohorts in response to clinical results and biomarker studies from preceding cohorts. Initially, 18 patients with HR+ *HER2*-mutant MBC received single-agent neratinib with a confirmed objective response rate (ORR) of 17% and progression-free survival (PFS) of 3.6 months.[9] Based on preclinical data suggesting synergy between neratinib and endocrine therapies,[8,11] a subsequent cohort tested neratinib and fulvestrant (N + F; N = 39 RECIST-evaluable patients). Combination therapy resulted in an ORR of 30% and prolongation of PFS to 5.4 months.[12] Notably, the independent MutHER clinical trial (NCT01670877) of N + F for HR+ *HER2*-mutant MBC reported similar results.[16]

In the SUMMIT and MutHER trials, genomic analysis of circulating tumor DNA (ctDNA) at baseline and progression revealed secondary *HER2* mutations and/or amplification as a putative mechanism of neratinib resistance.[12,16] No other genetic event was consistently observed, suggesting that a subset of *HER2*-mutant MBCs remained dependent on HER2 signaling upon disease progression.[19] Additionally, detection of *HER2* copy number amplification (CNA), or more than one *HER2* mutation at baseline, was associated with lack of clinical benefit in SUMMIT.[12] Enhancement of HER2 signaling associated with additional *HER2*-activating alterations suggested a need for combinations of HER2-targeted therapies to more completely inhibit HER2 signaling and maximize treatment response. Indeed, in MutHER, addition of trastuzumab in five patients at progression on N + F resulted in three responses and one long-term stable disease (SD).[16] Furthermore, neratinib plus trastuzumab in *HER2*-mutant cancer models led to greater inhibition of tumor growth and HER2 signaling than either agent alone.[15,20] We hypothesized that upfront treatment with triplet neratinib, fulvestrant, and trastuzumab (N + F + T) would prolong clinical benefit in patients with HR+ *HER2*-mutant MBC.

To test this hypothesis, a new SUMMIT cohort (N = 78) was opened in patients with HR+ HER2-negative *HER2*-mutant MBC. Prior cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) therapy was incorporated as an inclusion criterion for this study arm as CDK4/6i therapy had emerged as a standard of care for HR+ MBC. Under the advisement of regulatory authorities, a small, randomized sub-cohort (N = 21) comparing N + F + T versus F + T versus fulvestrant was included to isolate the contribution of neratinib. Genomic determinants of response, HER2 expression level, and mechanisms of acquired resistance were explored retrospectively via next-generation sequencing (NGS) of DNA and RNA from tissue and/or plasma samples, immunohistochemistry (IHC), and fluorescence in situ hybridization (FISH).

PATIENTS AND METHODS

Study design and treatment

The open-label, single-arm, multicohort, multi-tumor, phase II SUMMIT trial was conducted at 23 centers internationally, 15 of which enrolled 1 patient with breast cancer. The SUMMIT study has previously been described in detail.[9,12] Patients eligible

for inclusion in this cohort were aged 18 years with Eastern Cooperative Oncology Group performance status 0–2, histologically confirmed HR+ HER2-negative (institutionally reported HER2 IHC 0 or 1+, or IHC 2+/FISH non-amplified), advanced breast cancer with activating *HER2* mutation(s) assessed by local/institutional testing. All patients had received prior treatment with CDK4/6is. Eligibility for the study required a documented somatic activating *HER2* mutation, detected either from formalin-fixed paraffin-embedded (FFPE) tumor tissue or ctDNA by local testing carried out in a Clinical Laboratory Improvements Act or equivalent regionally certified laboratory. Central confirmation of *HER2* mutation was carried out retrospectively and concordance was evaluated. Key exclusion criteria were prior therapy with HER TKIs, cumulative epirubicin dose >900 mg/m^2 or cumulative doxorubicin dose >450 mg/m^2, and unstable brain metastases (treated and/or asymptomatic brain metastases were allowed).

Patients received N + F + T (oral neratinib 240 mg/day with mandatory loperamide prophylaxis for the first two cycles and as needed thereafter, intramuscular fulvestrant 500 mg on days 1, 15, and 29 of cycle 1 then every 4 weeks thereafter, and intravenous trastuzumab 8 mg/kg initially then 6 mg/kg every 3 weeks) or F + T (intramuscular fulvestrant 500 mg on days 1, 15, and 29 of cycle 1 then every 4 weeks thereafter, and intravenous trastuzumab 8 mg/kg initially then 6 mg/kg every 3 weeks) or fulvestrant (500 mg intramuscularly on days 1, 15, and 29, then once every 4 weeks thereafter). Patients were treated until disease progression, unacceptable toxicity, or withdrawal of consent. The protocol was approved by institutional review boards at all participating institutions; written informed consent was obtained for all patients before carrying out study-related procedures.

In a substudy, patients were randomized 1:1:1 to single-agent fulvestrant, F + T, or N + F + T, stratified by lines of prior therapy for metastatic disease (2 versus >2 lines) and prior fulvestrant therapy (yes versus no). Patients who initially received F + T or fulvestrant alone could cross over to the triplet upon clinical and/or radiologic progression (Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2023.08.003).

Tumor assessment

Tumor response was assessed locally according to RECIST (version 1.1) every 8 weeks by computed tomography or magnetic resonance imaging.[12] Adverse events (AEs) were classified according to Common Terminology Criteria for AEs (version 4.0) from consent until day 28 after discontinuation of study treatment.

Biomarker analysis

Central NGS.—Retrospective tissue NGS was conducted using either the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) or Tempus xT assays. Retrospective ctDNA sequencing of baseline, on-treatment, and end-of-treatment samples was conducted using either the Memorial Sloan Kettering-Analysis of Circulating cell-free DNA to Examine Somatic Status (MSK-ACCESS) or Tempus xF+ (Tempus Labs, Chicago, IL) cell-free DNA assays. Further information on these assays, Tempus mRNA analysis, and the Tempus deidentified multimodal database is provided in the Supplementary Methods, available at https://doi.org/10.1016/j.annonc.2023.08.003.

Central IHC and FISH.—Retrospective central HER2, ER, and progesterone receptor (PgR) IHC, and HER2 FISH were carried out on samples with sufficient FFPE tissue remaining after NGS. HER2, ER, and PgR IHC and HER2 FISH scores were determined according to manufacturer specifications [HercepTest™ and IQFISH pharmDx (Agilent Dako, Santa Clara, CA)]. HER2 IHC was further evaluated for H-score from original IHC images (Discovery Life Sciences GmbH, Kassel, Germany).

Statistical considerations

Description of the statistical analyses can be found in the Supplementary Methods, available at https://doi.org/10.1016/j.annonc.2023.08.003.

Preclinical experiments

Description of the *HER2*-mutant cell lines and immunoassays can be found in the Supplementary Methods, available at https://doi.org/10.1016/j.annonc.2023.08.003.

RESULTS

Patient characteristics

In total, 71 patients with HR+ *HER2*-mutant MBC with prior CDK4/6i therapy were enrolled between 27 February 2018 and 2 April 2022, and were assessable for efficacy. Twenty-one of the 71 patients comprised a small randomized, three-arm design intended to demonstrate the requirement for neratinib within the triplet; 7 patients received N + F + T, 7 received F + T, and 7 received fulvestrant alone. Efficacy of the triplet was evaluated in the 57 patients who received N + F + T upfront, i.e. 50 from the non-randomized cohort and 7 from the randomized cohort (Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2023.08.003).

Patients were extensively pretreated, with a median of 3 (range 1–10) prior lines of systemic therapy in the locally advanced/metastatic setting (Table 1). Twenty-seven patients (47%) had lobular breast cancer, higher than the previously reported incidence of 10%−15% for MBC and consistent with the enriched occurrence of *HER2* mutations in lobular versus ductal cancer.[1] In the randomized subset, 1/7 (14%), 2/7 (29%), and 5/7 (71%) patients had lobular cancer in the fulvestrant alone, F + T, and N + F + T groups, respectively.

The spectrum of *HER2* mutations (Supplementary Table S1 and Figure S2A, available at https://doi.org/10.1016/j.annonc.2023.08.003) was consistent with the expected mutation distribution in patients with breast cancer[21] and with prior MBC cohorts in SUMMIT.[12] The distribution of *HER2* mutations was also similar for ductal and lobular histologies (Supplementary Figure S2B, available at https://doi.org/10.1016/j.annonc.2023.08.003).

Efficacy

Among the 57 patients who received N + F + T, the investigator-assessed ORR [confirmed complete response (CR) or partial response (PR)] was 39% [95% confidence interval (CI) 26% to 52%], including 1 CR and 21 PRs (Figure 1A; Supplementary Table S2 and Figure S3, available at https://doi.org/10.1016/j.annonc.2023.08.003). The clinical benefit

rate (CBR; confirmed CR or PR, or SD 24 weeks) was 54% (N = 31/57). The median duration of response (DOR) was 14.4 months (95% CI 6.4–21.7 months) and the median PFS was 8.3 months (95% CI 6.0–15.1 months).

In the subset of seven patients randomized to N + F + T, the ORR was 29% (N = 2/7). No CRs or PRs were observed in either the fulvestrant monotherapy or F + T arms (Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2023.08.003). Four patients with disease progression on F + T crossed over to N + F + T; one of these patients subsequently had a confirmed PR (25%). Two of six patients (33%) with progression on fulvestrant and crossing over to N + F + T had a confirmed PR (Figure 1B).

Patients with ductal and lobular MBC experienced similar benefit from treatment with N + F + T (Table 2). Patients with lobular MBC had an ORR of 41% (95% CI 22% to 61%; N = 11/27), median DOR of 14.4 months (95% CI 5.0–21.7 months), CBR of 52% (95% CI 32% to 71%; N = 14/27), and median PFS of 8.3 months (95% CI 4.2–18.0 months). Those with ductal MBC had an ORR of 39% (95% CI 20% to 62%; N = 9/23), DOR of 14.3 months (95% CI 4.1 months-not estimable), CBR of 61% (95% CI 39% to 80%; N = 14/23), and median PFS of 8.3 months (95% CI 4.3–18.6 months).

ORRs were 63% (N = 5/8) for patients with V777L *HER2* kinase domain mutations, 24% (N = 4/17) for L755S, and 33% (N = 3/9) for those with other kinase domain missense mutations (Table 2). The ORR was 42% (N = 5/12) for patients with exon 20 insertion mutations and 33% (N = 1/3) for those with the extracellular domain S310F mutation, which stabilizes HER2-containing dimers and is the only *HER2* mutation reported to respond to HER2 antibody therapy.[22] Interestingly, despite patients whose tumors harbored L755S having a lower response rate, their median PFS was 15.1 months. Finally, 80% (N = 4/5) of patients with dual activating *HER2* mutations had a confirmed response.

Patients with prior CDK4/6i therapy and their outcomes according to prior CDK4/6i duration are described in the Supplementary Results, Supplementary Figure S4, and Table S4, available at https://doi.org/10.1016/j.annonc.2023.08.003.

Biomarkers

To standardize biomarker evaluation, pretreatment biopsies were centrally assessed retrospectively. The biomarker workflow is shown in Supplementary Figure S5, available at https://doi.org/10.1016/j.annonc.2023.08.003.

Central HER2 *mutation confirmation*

In total, 47 of 71 patients had sufficient tissue available for central NGS: 30 were fresh biopsies (taken <50 days before cycle 1, day 1) and 17 were archival. *HER2* mutation was centrally detected in 27/30 fresh biopsies (90%) and 16/17 archival tissues (94%). Central NGS was carried out on ctDNA for patients who did not have sufficient tissue (N = 20); of those, *HER2* mutation was detected in 8/10 patients (80%) enrolled on tissue biopsy and 8/10 (80%) enrolled on liquid biopsy (Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2023.08.003). Four patients did not have sufficient tissue or ctDNA for central NGS.

HER2 mutation was centrally detected in 48 of 57 patients who received N + F + T, in whom the ORR was 42% (20/48) (Table 3). An additional six patients had sufficient sample for central NGS but *HER2* mutation was not detected: none of those patients experienced response. *HER2* CNA was not detected in any tumor; low-level copy number 'gain' was detected in two patients (Supplemental Figure S6, available at https://doi.org/ 10.1016/j.annonc.2023.08.003). The remaining 3/57 patients did not have sufficient sample for central NGS.

Co-occurring genomic alterations

Co-mutations in *ERBB3* and/or *PIK3CA* have been associated with resistance to neratinib or N + F in patients with *HER2*-mutant MBC.[9,12,16,22] In the present study, the ORR for patients with co-mutation in *ERBB3* by central NGS was 40% (N = 4/10), with a median PFS of 25.7 months. Those with co-occurring *PIK3CA* mutation had a 21% ORR (N = 4/19) and median PFS of 7.8 months (Table 3). The ORR of patients with *ESR1* co-mutation was 50% (N = 3/6) and the median PFS was 8.3 months. *CDH1* mutation, a hallmark of lobular cancer, was detected in 27 patients; these patients had an ORR of 41% (11/27) and median PFS of 15.1 months. Patients with co-occurring *TP53* mutation, a general hallmark of poor prognosis,[23,24] had an ORR of 23% (3/13) and median PFS of 6.0 months. Mutation trends in other genes were not apparent (Supplementary Figure S6, available at https://doi.org/10.1016/j.annonc.2023.08.003).

HER2 expression

Post-enrollment, retrospective, central HER2 IHC and HER2 FISH were carried out on 32 and 30 patients, respectively, treated with N + F + T and with sufficient material for testing. Four patients had tissue centrally scored as IHC 0, 6 were IHC 1+, 21 were IHC 2+, and 1 was IHC 3+. Of those who were IHC 2+, the median FISH copy number was 3.6 (range 2.1–10.4) and the median central FISH ratio was 1.4 (range 1.0–5.0; Figure 1C). Central HER2 IHC or FISH status did not appear to be associated with response to N + F + T: patients with IHC 0/1+, 2+, or 3+ had ORRs of 20% (N = 2/10), 43% (N = 9/21), and 0% (N = 0/1), respectively. The ORR in patients whose breast cancer was FISH non-amplified was 40% (8/20) versus 30% (3/10) in those with central FISH-amplified disease (Figure 1D; Table 3). Patients with central FISH-amplified disease had to have been deemed HER2-negative at their local institutions to meet eligibility criteria. Analysis of centrally assessed HER2 mRNA expression is described in the Supplementary Results, and Supplementary Figure S7 available at https://doi.org/10.1016/j.annonc.2023.08.003.

Mechanisms of acquired resistance

Detection of additional *HER2* mutations and/or HER2 CNA[12] upon progression on neratinib or N + F prompted us to test combination therapy with trastuzumab as an additional arm of SUMMIT. Thus, we carried out longitudinal ctDNA sequencing to evaluate whether the same or alternative mechanisms of acquired resistance were observed in patients progressing on dual HER2 targeting. Eight patients with a PR had evaluable ctDNA from liquid biopsies at enrollment (i.e. before treatment), while on treatment, and at progression; an additional three patients (two PR and one CR) remained on treatment at the time of the last sequenced blood draw. In all cases, the variant allele frequency (VAF) of the enrollment

HER2 mutation, along with secondary *HER2* mutations in three cases, decreased upon treatment with N + F + T (Figure 2A and B). Notably, the enrollment *HER2* mutation became undetectable following treatment in six of eight patients and remained undetectable at the last blood draw in the three patients still on treatment (Figure 2A and B). Both patients whose enrollment *HER2* mutations did not become undetectable during treatment had L755S mutations (Figure 2A). Upon progression, re-emergence of the enrollment *HER2* mutation was observed, coincident with apparent acquisition of additional *HER2* mutations in three patients, including the gatekeeper T798I mutation, and several variants of unknown significance (L786V, D873N, and D582N; Figure 2A and C). Two patients acquired *PTEN* Q214* and *PIK3CA* H1047L mutations and three acquired *TP53* mutations following N + F + T treatment (Figure 2C).

Longitudinal ctDNA analysis was also carried out for two patients who experienced PR upon cross-over to N + F + T after progression on fulvestrant (Figure 3A) and F + T (Figure 3B). In both cases, decreases in VAFs for ctDNA mutations were observed after cross-over, coinciding with imaging response. The patient illustrated in Figure 3B enrolled in SUMMIT based on detection of *HER2* G776V (0.04%) by ctDNA analysis following disease progression after 10 months on letrozole and palbociclib. On central ctDNA testing, *HER2* G776V was not detected in the baseline sample, possibly because of its low VAF. On treatment with F + T, this patient experienced SD, with 4 months' PFS. After switching to N + F + T, she experienced PR, with a PFS of 8 months. Upon progression on N + F + T, she acquired *HER2* T798I (0.3% VAF) and several *PIK3CA* mutations including E545K (3%), E542K (2%), and E545A (0.06%), which were not detected before enrollment. Based on ctDNA results at progression, she was subsequently treated with fulvestrant plus the phosphoinositide 3-kinase (PI3Ka) inhibitor alpelisib, on which she achieved PR (Figure 3B).

Preclinical rationale for dual HER2 targeting

Mutant *HER2* exhibits enhanced dimerization with the HER3 co-receptor, with enhanced PI3K/AKT/mTOR signaling.[8] Addition of trastuzumab to neratinib is predicted to block this heterodimerization[25] and potentially enhance inhibition of PI3K/AKT/mTOR signaling and antitumor activity. We explored this possibility using MCF7 cells engineered to express *HER2* V777L via knock-in of the mutant allele at the endogenous locus. Treatment of MCF7 *HER2* V777L cells with 100 nM neratinib resulted in strong and sustained inhibition of phosphorylated HER2 for up to 72 h. Phosphorylated HER3, phosphorylated AKT, and phosphorylated S6 levels were also markedly inhibited in *HER2*-mutant cells, although they began to recover after 24 h. Co-treatment with trastuzumab abrogated recovery of HER3 and AKT phosphorylation (Supplementary Figure S8, available at https://doi.org/10.1016/j.annonc.2023.08.003), providing a rationale for enhanced activity with the combination. These data, combined with acquisition of *HER2* gene amplifications following treatment of *HER2*-mutant tumors with N + F,[12] support adding trastuzumab to the combination.

Safety

The most common treatment-emergent AEs are summarized in Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2023.08.003. Diarrhea of any grade occurred in

93% (N = 53/57) of patients who received N + F + T, in 29% (N = 2/7) of those who received F + T, and in none of those who received fulvestrant alone. Grade 3 diarrhea occurred in 53% (N = 30/57) of patients in the N + F + T group and was not observed in the F + T or fulvestrant monotherapy groups. For N + F + T, grade 3 diarrhea typically occurred early [median time to grade 3 diarrhea was 8 days; interquartile range (IQR) 4–32 days] after the start of treatment and was transient (median cumulative duration 4 days; IQR: 2–10 days). Among patients with diarrhea, 27 and 13 were managed by dose interruptions and dose reductions, respectively; only 2 patients withdrew from treatment.

DISCUSSION

The SUMMIT trial has provided a clinical platform for evaluating HER2 TKI-containing therapies for patients with MBC whose tumors harbor *HER2* mutations. We speculated that dual HER2 targeting, through addition of trastuzumab to N + F, might prevent or delay emergence of additional *HER2* genomic alterations and lengthen responses observed with the doublet. This was further supported by preclinical studies using breast cancer cells expressing *HER2* V777L, suggesting that addition of trastuzumab to neratinib monotherapy prolonged suppression of HER3 phosphorylation. Indeed, DOR, CBR, and PFS were all superior in patients treated with N + F + T versus N + F. Of note, all patients treated with N + F + T had progressed on prior CDK4/6i. Neratinib appeared to be a critical component of the combination, as demonstrated by lack of response in the small cohorts of patients treated with fulvestrant alone or F + T, and subsequent response in patients who crossed over to N + F + T after progression on fulvestrant alone or F + T.

Although we recognize that cross-trial and cross-cohort comparisons must be interpreted with caution, treatment with N + F + T appeared to be superior to N + F in patients with HR+ *HER2*-mutant MBC. Firstly, responses to N + F + T were observed in patients with ductal and lobular histology, as opposed to the previously reported association of lobular histology with response to N + F.[16] Notably, the incidence of lobular breast cancer was higher in our cohort than previously reported for MBC, but consistent with enrichment for *HER2* mutations in patients with lobular versus ductal breast cancers.[1] Secondly, four of five patients with tumors harboring more than one activating *HER2* mutation experienced a confirmed response, in contrast to previous association of this characteristic with lack of clinical benefit to N + F.[12] Thirdly, co-occurrence of *HER2* and *ERBB3* mutations did not preclude response to N + F + T, in contrast to neratinib alone or N + F.[12] Additionally, >20% of patients with co-occurring *PIK3CA* mutation responded to treatment, suggesting that dual HER2 targeting may block the enhanced PI3K signaling in these tumors. Finally, the low response rate and high median PFS in patients whose tumors harbored L755S were consistent with both the indolence and reduced sensitivity to neratinib of L755S compared with other activating *HER2* mutations in preclinical models.[7,22,26–28]

The finding that most HER2-mutant MBC samples were IHC 2+ is consistent with a recent genomic description of HER2-low breast cancers, in which HER2-mutant samples were exclusive to the HER2 IHC 2+/copy number-equivocal (HLBC-2E) group and in fact comprised 14% of that group. Indeed, HER2 mRNA levels in *HER2*-mutant tumors from SUMMIT patients with available tissue and those in a clinicogenomic/transcriptomic

database of >4000 patients with HR+ MBC[29] were midway between HER2-positive and HER2-negative disease. We thus speculate that HR+ *HER2*-mutant tumors may fall broadly into the newly described 'HER2-low' classification.[30,31] At the time of writing, data were not available for patients with *HER2* mutations from the DESTINY-Breast04 clinical trial (ClinicalTrials.gov Identifier: NCT03734029), which measured the efficacy of trastuzumab deruxtecan (T-DXd) in patients with HER2-low MBC. If results are positive, this may enable exploration of sequencing of antibody–drug conjugates (ADCs) and TKIs targeting HER2. In preclinical experiments, neratinib induced HER2 receptor ubiquitination and endocytosis;[32] thus, combining neratinib with a HER2 ADC may increase ADC internalization. Further, combining neratinib with trastuzumab emtansine or with T-DXd (T-DM1) in *HER2*-mutant breast and lung cancer patient-derived xenografts resulted in synergistic growth inhibition in preclinical models.[26,33] A phase II study of T-DXd in *HER2*-mutant solid tumors has recently completed enrollment (ClinicalTrials.gov identifier: NCT04639219), and a phase I clinical trial exploring the safety and tolerability of neratinib + T-DXd in HER2-altered breast cancer is currently underway (ClinicalTrials.gov identifier: NCT05372614).

Serial ctDNA sequencing enables an individualized approach to cancer treatment. We observed that *HER2* mutation VAFs decreased to below the level of detection upon treatment in most responders with emergence of additional *HER2* mutations upon progression, *TP53* mutations, and/or mutations in downstream effectors of HER2 signaling (*PIK3CA, PTEN*), consistent with prior cohorts.[9,12,34] Supporting the requirement of neratinib in the combination, one patient initially randomized to F + T had a concomitant increase in VAFs and emergence of *HER2* CNA. Upon addition of neratinib, *HER2* amplification became undetectable, and the patient responded for 6.2 months. Furthermore, ctDNA sequencing upon progression revealed acquisition of an activating *PIK3CA* mutation, which responded to alpelisib. These cases highlight that N + F + T can be effective for patients who acquire *HER2* mutations at disease progression on a CDK4/6i, and that *PIK3CA* mutations could represent acquired resistance mechanisms to N + F + T that could potentially be further treated with PI3K inhibitors. These cases also highlight the potential utility of serial ctDNA in sequentially tailoring cancer treatment in response to individual tumor evolution.

The overall safety profile of neratinib for patients with HR+, *HER2*-mutant MBC was consistent with prior studies, although the rate of grade 3 diarrhea (53%) in patients who received the triplet combination of N + F + T was higher than that observed in other SUMMIT breast cohorts of either neratinib monotherapy (26.5%) or N + F (23.4%),[12] in the N + F cohort of the MutHER clinical trial (25%),[16] and also higher than that in a phase I/II study of N + T (15.6%)[35] or a phase I study of N + T + paclitaxel in HER2-positive MBC (0%).[36] Studies of other neratinib combinations with recommended or mandatory antidiarrheal prophylaxis in MBC have also reported lower rates of grade 3 diarrhea: 24% with neratinib plus capecitabine in the phase III NALA trial,[37] 23% with neratinib + T-DM1 in the TBCRC 022 trial,[38] and 30% with neratinib plus paclitaxel in the NEfERT-T trial.[39] The reason for increased grade 3 diarrhea within this cohort remains unclear; it is possible that the N + F + T triplet increases diarrhea, although no mechanism for such an effect has been reported. Importantly, over half of the patients in this cohort were enrolled during the peak of the COVID-19 pandemic, and we speculate that close monitoring for compliance

with loperamide prophylaxis may have been compromised during that time. Of note, 2-week dose escalation of neratinib with loperamide as needed is now recognized as an optimal diarrhea management strategy and included in the US prescribing information and National Comprehensive Cancer Network (NCCN) guidelines based on results from the phase II CONTROL trial, which demonstrated a grade 3 diarrhea rate of 13.3%.[40] Dose escalation was not used in the present study but is now recommended for use in the clinical setting.

In conclusion, these data represent completion of the hypothesis-generating SUMMIT basket trial, which supports a model of the Nimble trial adaptation in response to translational findings. N + F + T showed encouraging clinical efficacy in heavily pretreated patients with HR+ breast cancer harboring *HER2*-activating mutations. In line with this result, neratinib-based combinations have recently been endorsed for this molecularly defined population of patients by NCCN guidelines. Furthermore, 16 patients enrolled in this trial exhibited a *HER2* mutation in DNA retrieved from archival biopsy, confirming these pathogenic alterations are present much earlier in tumor evolution. This implies that with future increased early utilization of NGS, and thus increased detection of *HER2* mutations at the time of breast cancer diagnosis or at endocrine resistance,[10] the results presented herein support future testing of neratinib-based combinations in patients with HR+ *HER2*-mutant early breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA SHARING

The authors declare that the data supporting the findings of this study are available within the article. Qualified researchers and study participants may submit requests for other study documentation and clinical trial data to clinicaltrials@pumabiotechnology.com for consideration.

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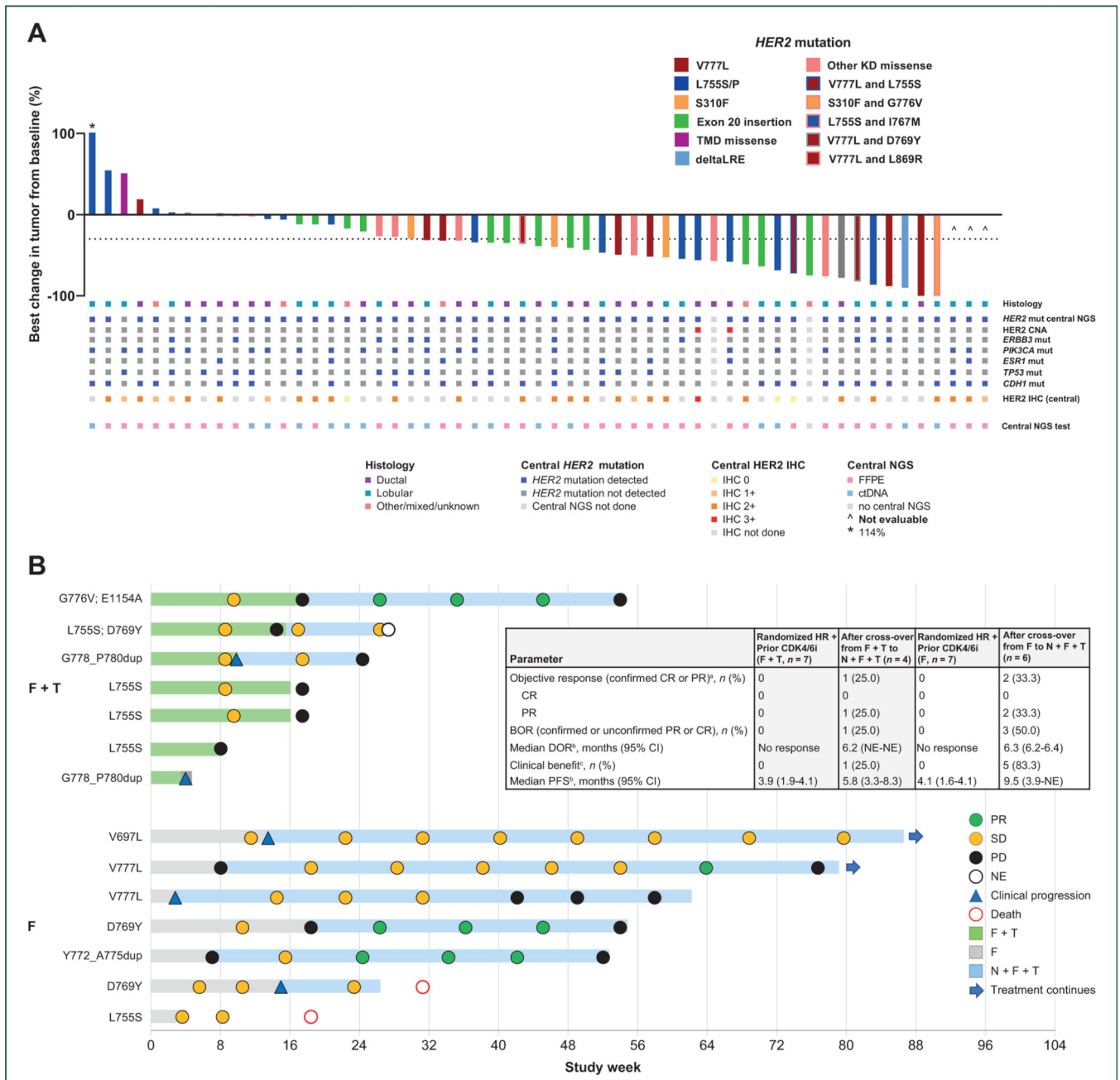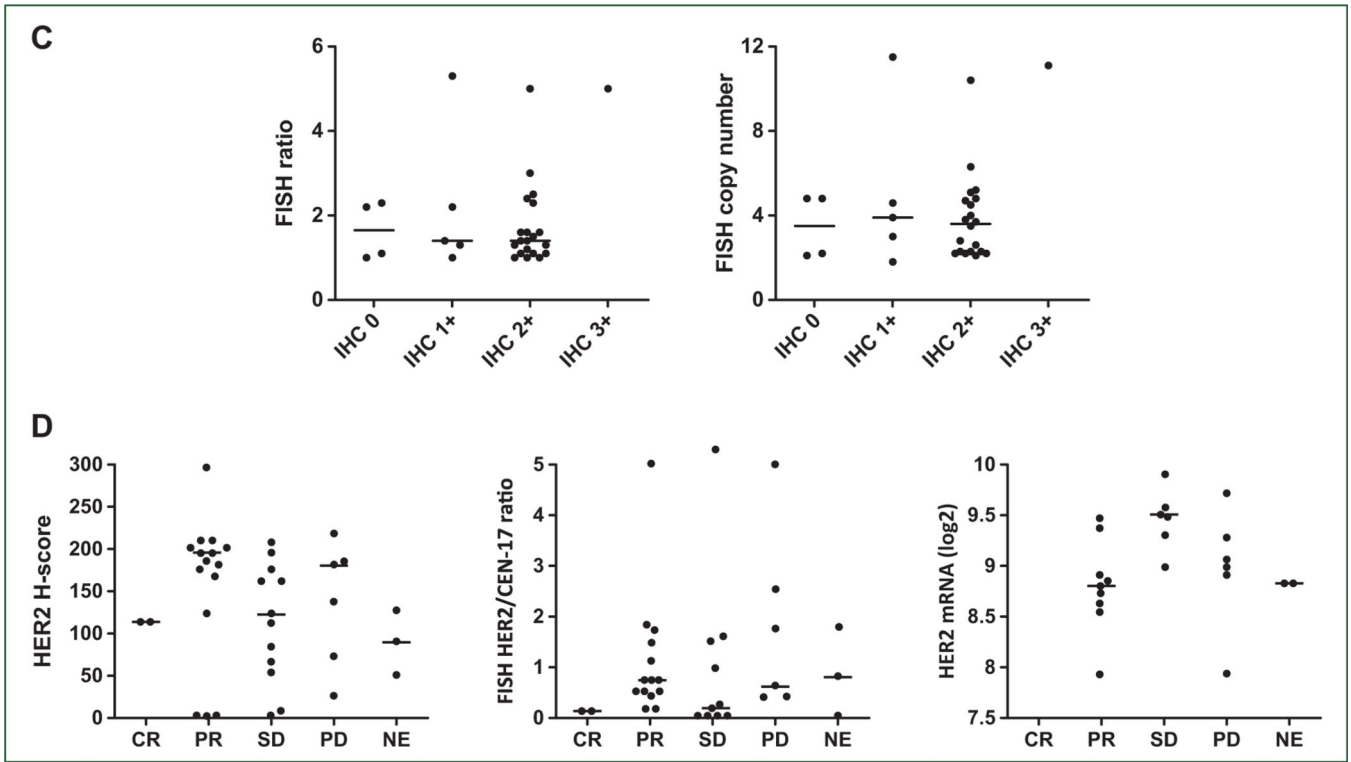
Figure 1. (A) Best change in tumor from baseline and corresponding histology and central biomarker analysis. (B) Duration of treatment and best response in patients randomized to F + T or F, before and after cross-over to N + F + T. (C) HER2 expression in patients treated with N + F + T. (D) HER2 H-score, FISH ratio, or mRNA expression, and response to N + F + T. The solid horizontal line represents the median.

BOR, best overall response; CEN-17, centromere of chromosome 17; CI, confidence interval; CDK4/6i, cyclin-dependent kinase 4/6 inhibitor; CNA, copy number amplification; CR, complete response; ctDNA, circulating tumor DNA; dup, duplication; F, fulvestrant; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; KD, kinase domain; mut, mutation; N, neratinib; NE, not evaluable; NGS, next-generation sequencing; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; T, trastuzumab; TMD, transmembrane domain.

[a]Objective response defined as either a CR or PR that is confirmed no less than 4 weeks after the criteria for response are initially met.

[b]Kaplan–Meier analysis. For cross-over patients, calculated from time of cross-over to N + F + T.

[c]Clinical benefit is defined as confirmed CR or PR or SD for 24 weeks (within a ±7-day visit window).
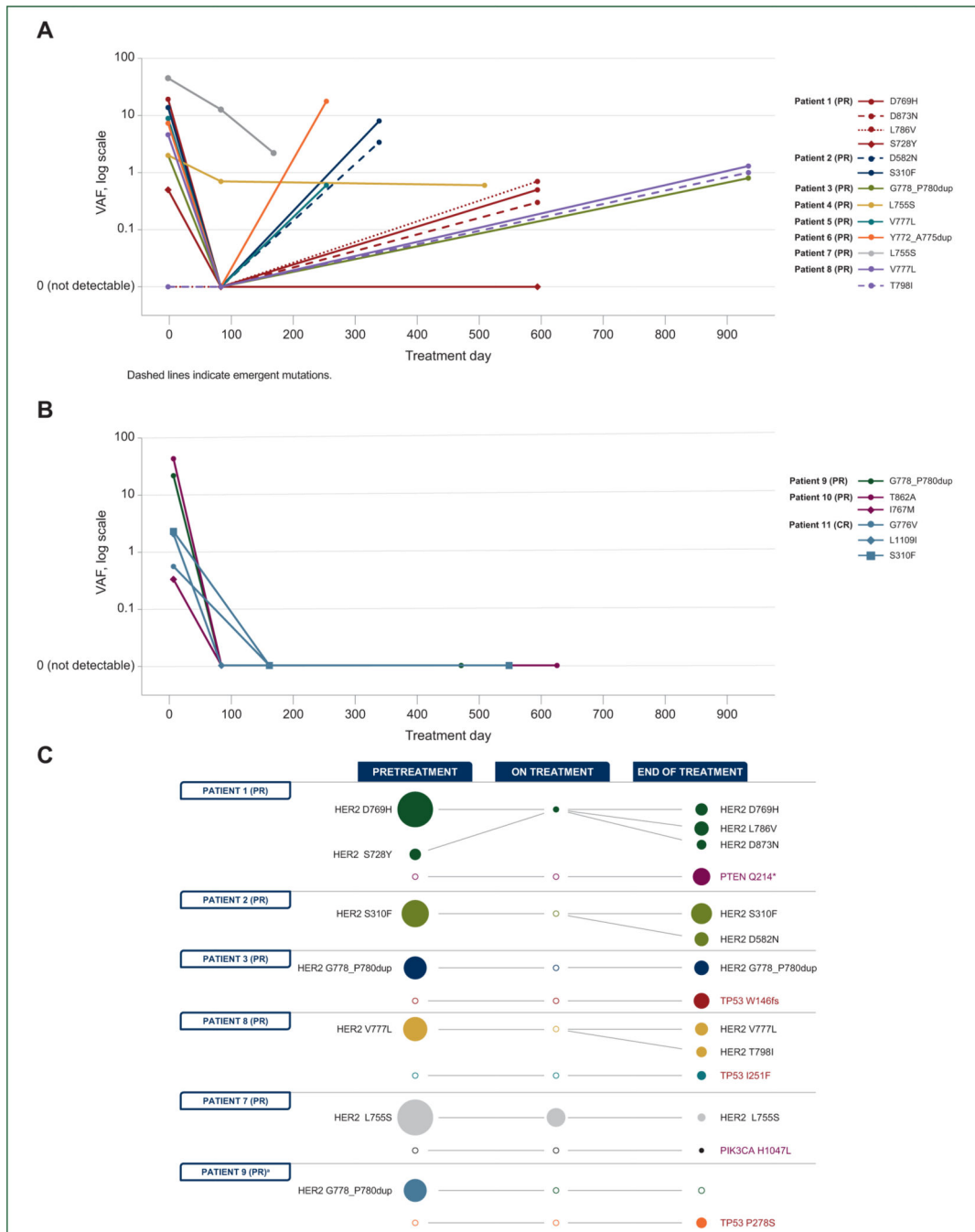
Figure 2. Longitudinal *HER2* mutation VAFs in patients with response to N + F + T.
Blood draw and ctDNA sequencing: (A) before treatment, on treatment, and at the end of treatment in patients who progressed; and (B) before treatment and in two on-treatment samples for patients who had not yet progressed. Solid and dotted lines represent original and emergent mutations, respectively. (C) Prevalence of notable mutations throughout the course of treatment with N + F + T; size of the bubble corresponds with VAF. Empty circle indicates mutation not detectable.

CR, complete response; ctDNA, circulating tumor DNA; dup, duplication; F, fulvestrant; N, neratinib; PR, partial response; T, trastuzumab; VAF, variant allele frequency.

[a]Patient remained on treatment as of data cut-off.

Figure 3. Cross-over case studies.

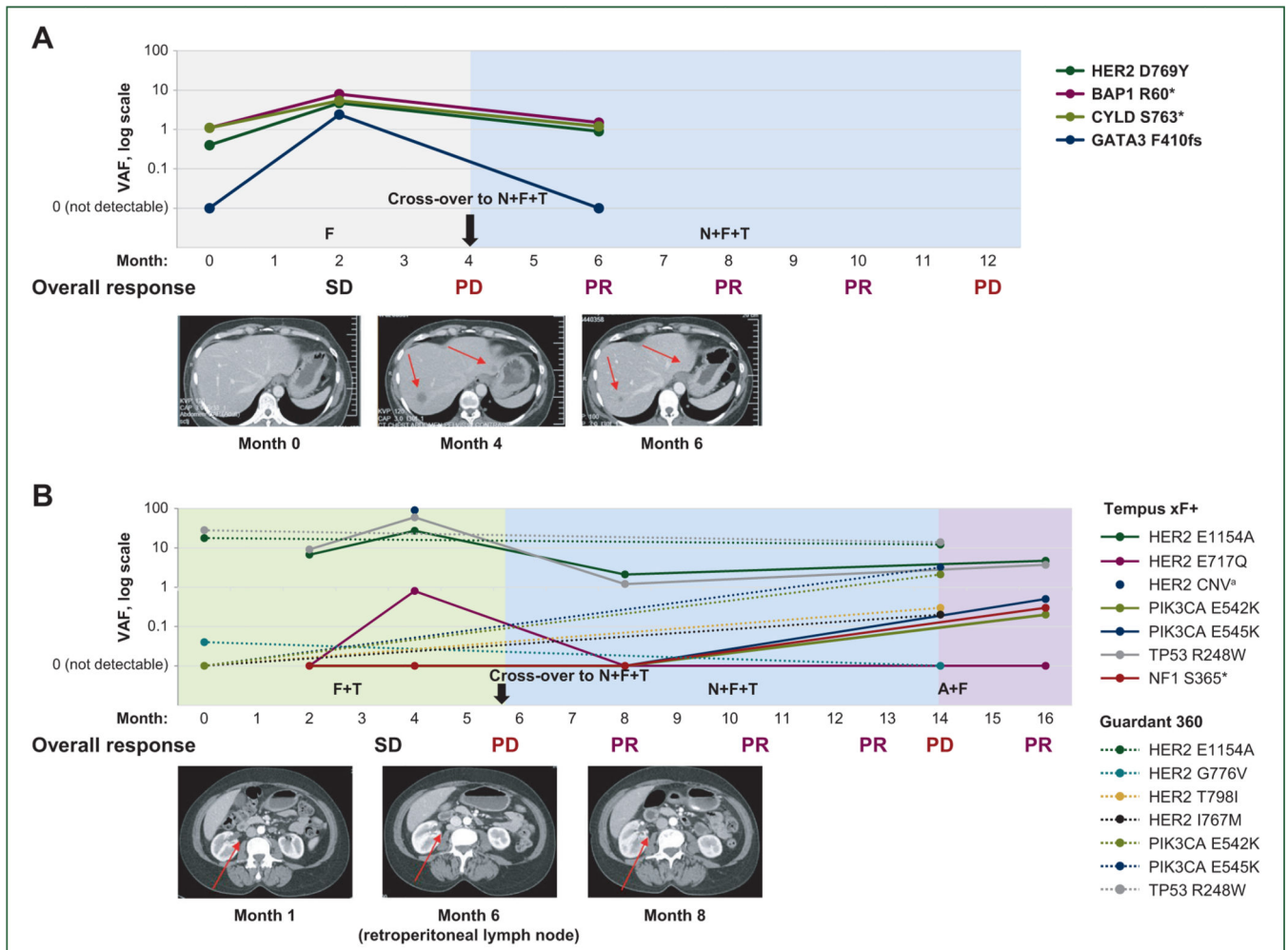(A) patient crossed over from F to N + F + T. The patient was enrolled on tissue-based NGS (FoundationOne CDx), which reported HER2 D769Y. The patient was initially randomized to F alone; upon progression, she crossed over to receive N + F + T. *HER2* D769Y VAF increased upon initial treatment with F then decreased upon cross-over. (B) Patient crossed over from F + T to N + F + T. The patient was enrolled on ctDNA (Guardant 360), which detected *HER2* G776V at 0.04% VAF. *HER2* E1145 and E717Q, but not G776V, were centrally detected in pretreatment ctDNA. The patient was initially randomized to F + T; upon progression she crossed over to receive N + F + T. *HER2* VAFs of both mutations increased upon initial treatment with F + T, then decreased upon addition of N. *HER2* copy number gain was also detected upon initial treatment, but not after the patient crossed over to receive the triplet. Upon progression to N + F + T, the *HER2* T798I gatekeeper mutation emerged, along with PIK3CA-activating mutations E542K and E545K. After progression on N + F + T, the patient was treated with A + F and experienced a PR.

A, alpelisib; CNV, copy number variation; ctDNA, circulating tumor DNA; F, fulvestrant; N, neratinib; NGS, next-generation sequencing; PD, progressive disease; PR, partial response; SD, stable disease; T, trastuzumab; VAF, variant allele frequency.

[a]*HER2* CNV only detected at second timepoint. Solid lines indicate Tempus xF+ results; dotted lines indicate Guardant360 results.

Table 1.

Baseline demographics and characteristics

Characteristics	Non-randomized + randomized HR+, prior CDK4/6i (N + F + T, N = 57)	Randomized HR+, prior CDK4/6i (F + T, n = 7)	Randomized HR+, prior CDK4/6i (F, n = 7)	Randomized HR+, prior CDK4/6i (N + F + T, n = 7)
Median age, years (range)	59.0 (25–83)	65.0 (37–72)	55.0 (46–80)	57.0 (44–77)
Sex, n (%)				
Female	56 (98)	7 (100)	7 (100)	7 (100)
Male	1 (2)	0	0	0
Menopausal status, n (%)				
Post-menopausal	49 (86)	7 (100)	7 (100)	7 (100)
Pre-menopausal	7 (12)	0	0	0
NA	1 (2)	0	0	0
ECOG performance status, n (%)				
0	27 (47)	4 (57)	5 (71)	3 (43)
1	29 (51)	3 (43)	2 (29)	4 (57)
2	1 (2)	0	0	0
Histological type, n (%)				
Ductal	23 (40)	5 (71)	5 (71)	2 (29)
Lobular	27 (47)	2 (29)	1 (14)	5 (71)
Mixed ductal and lobular	1 (2)	0	0	0
Other	6 (11)	0	1 (14)	0
Location of disease at time of enrollment, n (%)				
Visceral	51 (89)	6 (86)	7 (100)	7 (100)
Non-visceral only	5 (9)	1 (14)	0	0
Missing	1 (2)	0	0	0
Median time from first metastasis to enrollment, years (range)	2.3 (0–15)	1.0 (0–4)	1.6 (0–4)	2.1 (1–7)
Prior treatment for locally advanced/metastatic disease, n (%)	56 (98)[a]	7 (100)	7 (100)	7 (100)
Median no. of prior anticancer regimens (range)	3 (1–10)	2 (1–10)	2 (1–6)	2 (1–6)
Prior endocrine therapy, n (%)	56 (98)	6 (86)	7 (100)	7 (100)
Aromatase inhibitor	33 (58)	5 (71)	5 (71)	5 (71)

Characteristics	Non-randomized + randomized HR+, prior CDK4/6i (N + F + T, N = 57)	Randomized HR+, prior CDK4/6i (F + T, n = 7)	Randomized HR+, prior CDK4/6i (F, n = 7)	Randomized HR+, prior CDK4/6i (N + F + T, n = 7)
Fulvestrant	44 (77)	3 (43)	4 (57)	5 (71)
Tamoxifen	7 (12)	1 (14)	0	0
Prior chemotherapy, n (%)	34 (60)	2 (29)	4 (57)	3 (43)
Prior HER2 antibody-directed therapy, n (%)	5 (9)	1 (14)	1 (14)	1 (14)
Prior CDK4/6i, n (%)	54 (95)	7 (100)	7 (100)	7 (100)
Prior PIK3CAi, n (%)	7 (12)	1 (14)	1 (14)	1 (14)
Prior mTORi, n (%)	16 (28)	0	1 (14)	1 (14)

CDK4/6i, cyclin-dependent kinase 4/6 inhibitor; ECOG, Eastern Cooperative Oncology Group; F, fulvestrant; HR+, hormone receptor-positive; mTORi, mTOR inhibitor; N, neratinib; NA, not applicable; PIK3CAi, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha inhibitor; T, trastuzumab.

[a]Missing information for one patient.

Table 2.

Efficacy by histology or enrollment *HER2* mutation in patients treated with neratinib + fulvestrant + trastuzumab

Outcome	Histology			Enrollment *HER2* mutation							
	Lobular (*n* = 27)	Ductal (*n* = 23)	Other/ mixed/ unknown (*n* =7)	L755S (*n* = 17)	Exon 20 insertion (*n* = 12)	Other KD missense (*n* = 9)	V777L (*n* = 8)	S310F (*n* = 3)	TMD missense (*n* = 2)	Multiple mutations[a] (*n* = 5)	Exon 19 deletion (*n* = 1)
Objective response[b], *n* (%)	11 (41)	9 (39)	2 (29)	4 (24)	5 (42)	3 (33)	5 (63)	1 (33)	0	4 (80)	0
CR	1 (4)	0	0	0	0	0	0	0	0	1 (20)	0
PR	10 (37)	9 (39)	2 (29)	4 (24)	5 (42)	3 (33)	5 (63)	1 (33)	0	3 (60)	0
Best overall response (confirmed or unconfirmed PR or CR), *n* (%)	14 (52)	13 (57)	2 (29)	5 (29)	7 (58)	5 (56)	6 (75)	1 (33)	0	4 (80)	1 (100)
Median DOR[c], months (95% CI)	14.4 (5.0–21.7)	14.3 (4.1-NE)	NE	14.3 (11.1–21.7)	NE	6.4 (5.0–18.6)	NE	8.2 (NE-NE)	NE	16.0 (6.0–16.0)	NE
Clinical benefit[d], *n* (%)	14 (52)	14 (61)	3 (43)	9 (53)	6 (50)	4 (44)	5 (63)	1 (33)	0	5 (100)	1 (100)
Median PFS[c], months (95% CI)	8.3 (4.2–18.0)	8.3 (4.3–18.6)	NE (NE-NE)	15.1 (2.6–25.7)	10.2 (4.3-NE)	7.0 (2.0–8.5)	6.1 (1.9-NE)	3.4 (1.9–10.2)	1.8 (NE-NE)	18.0 (8.3–18.0)	12.7 (NE-NE)

Data cut-off: 21 October 2022. Tumor response based on investigator tumor assessments (RECIST version 1.1).

CI, confidence interval; CR, complete response; DOR, duration of response; KD, kinase domain; NE, not estimable; PFS, progression-free survival; PR, partial response; TMD, transmembrane domain.

[a] S310F/G776V, V777L/D769Y, G776S/H878Y, L755S/V777L, L755S/I767M/L786V.

[b] Objective response defined as either a CR or PR that is confirmed 4 weeks after the criteria for response are initially met.

[c] Kaplan–Meier analysis.

[d] Clinical benefit is defined as confirmed CR or PR, or stable disease for 24 weeks (within a ±7-day visit window).

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Table 3.

Efficacy by centrally assessed exploratory biomarker in patients treated with neratinib + fulvestrant + trastuzumab

Centrally assessed exploratory biomarker	No. of patients	ORR, *n* (%)	CBR, *n* (%)	Median PFS, months (95% CI)
NGS mutation				
HER2				
Yes	48	20 (42)	28 (58)	10.2 (6.1–18.6)
No	6	0	0	4.2 (1.8–6.2)
Insufficient sample	3	2 (67)	3 (100)	12.7 (8.2–12.7)
HER2 and *ERBB3*	10	4 (40)	6 (60)	25.7 (1.0-NE)
HER2 and *ESR1*	6	3 (50)	4 (67)	8.3 (1.9–18.6)
HER2 and *CDH1*	27	11 (41)	15 (56)	15.1 (2.4–18.6)
HER2 and *TP53*	13	3 (23)	5 (38)	6.0 (1.9–8.3)
HER2 and *PIK3CA*	19	4 (21)	10 (53)	7.8 (2.2–10.2)
HER2 and none of above	7	4 (57)	4 (57)	NE (3.9-NE)
IHC category				
0/1+	10	2 (20)	4 (40)	7.0 (1.8-NE)
2+	21	9 (43)	12 (57)	8.3 (2.6–18.0)
3+	1	0	0	3.9 (NE-NE)
Insufficient tissue	25	11 (44)	15 (60)	10.2 (4.3–18.6)
FISH category				
Amplified	10	3 (30)	5 (50)	6.1 (1.0-NE)
Non-amplified	20	8 (40)	11 (55)	8.5 (4.1–18.0)
Insufficient tissue	27	11 (41)	15 (56)	8.3 (4.2–18.6)
Overall HER2 status (IHC 3+ or FISH amplified)				
Positive	10	3 (30)	5 (50)	6.1 (1.0-NE)
Negative	20	8 (40)	11 (55)	8.5 (4.1–18.0)
Insufficient tissue	27	11 (41)	15 (56)	8.3 (4.2–18.6)
Molecular subtype				
Luminal A	4	0	1 (25)	3.1 (1.0-NE)
Luminal B	4	2 (50)	2 (50)	14.0 (2.0–25.7)
HER2 enriched	11	5 (45)	8 (73)	8.3 (1.9–10.2)

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Centrally assessed exploratory biomarker	No. of patients	ORR, n (%)	CBR, n (%)	Median PFS, months (95% CI)
Insufficient tissue	38	15 (39)	20 (53)	8.3 (4.7–18.0)

CBR, clinical benefit rate; CI, confidence interval; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NE, not estimable; NGS, next-generation sequencing; ORR, objective response rate; PFS, progression-free survival.