Genomic analysis of circulating tumor DNA from patients with hormone receptor-positive, *HER2*-mutant metastatic breast cancer enrolled in SUMMIT: Mechanisms of acquired resistance to neratinib + fulvestrant + trastuzumab

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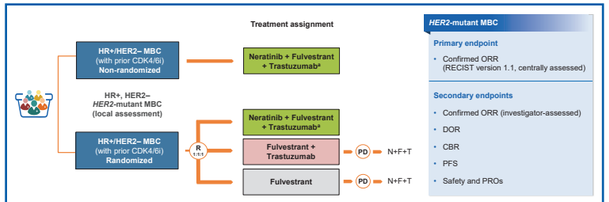
Introduction

- *HER2* mutations are oncogenic drivers in a subset of metastatic breast cancers (MBCs) and may be acquired as a mechanism of resistance to endocrine therapy.[1–4]
- Neratinib (N) is an oral, irreversible, pan-HER tyrosine kinase inhibitor with demonstrated preclinical and clinical activity against *HER2*-mutant cancers.[1–8]
- In the hypothesis-generating SUMMIT basket trial (NCT01953926), original cohorts of patients with locally assessed hormone receptor-positive (HR+), HER2-negative (HER–), *HER2*-mutant MBC received N alone or in combination with fulvestrant (N+F; Figure 1). Clinical responses were promising but of short duration. Clinical progression coincided with emergence of additional *HER2* mutations and/or amplification of the mutant allele.[6]
- Addition of trastuzumab (T) to the doublet was postulated to prolong response. The combination of N+F+T in heavily pretreated patients with HR+, *HER2*-mutant MBC who had received cyclin-dependent kinase 4/6 inhibitors (CDK4/6is; n=51) yielded a confirmed overall response rate (ORR) of 35.3%, median duration of response (DOR) of 14.3 months, clinical benefit rate (CBR) of 47.1%, and median progression-free survival (PFS) of 8.2 months.[8]
- Seven of these 51 patients were part of a cohort that was randomized (1:1:1) to N+F+T, F+T, or F alone. Patients randomized to F+T or F could crossover to N+F+T upon progression. No patients responded to F or F+T; however, one of four patients who crossed over to N+F+T after progressing on F+T responded to the triplet, as did two of six who crossed over after progressing on F.[6]
- We undertook longitudinal circulating tumor DNA (ctDNA) sequencing in patients who responded to N+F+T upfront and after crossover.

Figure 1. SUMMIT study design: HR+, HER2–, *HER2*-mutant MBC cohorts



[a]Loperamide prophylaxis: oral 12 mg days 1–14, 8 mg days 15–18; as needed thereafter.
PD, progressive disease; PRO, patient-reported outcome; R, randomization; RECIST, Response Evaluation Criteria in Solid Tumors.

ClinicalTrials.gov Identifier NCT01953926.

Objectives

- To report baseline *HER2* alterations, as assessed by central next-generation sequencing (NGS) of ctDNA and compare with reported enrollment mutations.
- To longitudinally evaluate *HER2* mutation variant allele frequencies (VAFs) in patients with clinical responses to N+F+T at three timepoints: before treatment, on treatment, and either at end of treatment, progression, or at last blood draw.
- To assess longitudinal genomic profiles of patients randomized to F or F+T who then crossed over to N+F+T.
- To determine whether potential mechanisms of acquired resistance to N+F+T (dual HER2 therapy) are consistent with or different from those previously reported for N+F.

Methods

- NGS was conducted using the Tempus xF+ assay (Tempus Labs, Chicago, IL)
  - Tempus xF+ is a targeted liquid biopsy panel that detects cell-free DNA (cfDNA) in blood specimens obtained from patients with advanced solid tumors and detects:
    - Single-nucleotide variants and insertions and/or deletions in 523 genes.
    - Gene rearrangements in 10 genes.
    - Copy number variants (CNVs), including gains in seven genes and losses in two genes.

Analysis cohort

- Patients were enrolled on SUMMIT on the basis of an activating *HER2* mutation as reported by any commercial or Clinical Laboratory Improvement Amendments/College of American Pathologists (or regionally equivalently) certified laboratory, sequenced from either tissue (formalin-fixed paraffin embedded; FFPE) or liquid biopsy.
- A total of 68 patients had HR+, HER2–, *HER2*-mutant MBC and prior CDK4/6i therapy; ctDNA was centrally assessed for 24 patients (Figure 2).
- ctDNA from pre-treatment liquid biopsies was sequenced by Tempus xF+ (Table 2). The genomic spectrum was consistent with prior SUMMIT cohorts and with publicly available datasets (Figure 3).

Results

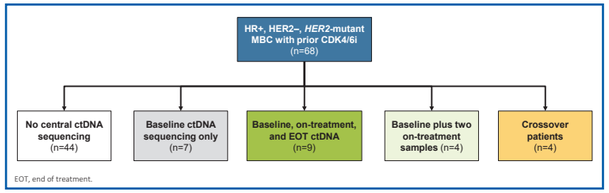
- Patients with HR+, HER2–, *HER2*-mutant MBC treated with N+F+T had increased response and prolonged PFS (Table 1).
- Small randomized cohorts supported the contribution of N to the triplet.

Table 1. Efficacy summary overall and according to treatment received[8]

Parameter	Non-randomized + Randomized HR+ Prior CDK4/6i (N+F+T, n=51)	Randomized HR+ Prior CDK4/6i (F+T, n=7)	After crossover from F+T to N+F+T (n=4)	Randomized HR+ Prior CDK4/6i (F, n=7)	After crossover from F to N+F+T (n=6)
Objective response (confirmed CR or PR)[a], n (%)	18 (35.3)	0	1 (25.0)	0	2 (33.3)
CR	1 (2.0)	0	0	0	0
PR	17 (33.3)	0	1 (25.0)	0	2 (33.3)
Best overall response[b] (confirmed or unconfirmed PR or CR), n (%)	25 (49.0)	0	1 (25.0)	0	2 (33.3)
Median DOR[c], months (95% CI)	14.3 (6.4–NE)	No response	6.2 (NE–NE)	No response	6.3 (6.2–6.4)
Clinical benefit[d], n (%)	24 (47.1)	0	1 (25.0)	0	5 (83.3)
Median PFS[c], months (95% CI)	8.2 (4.7–12.7)	3.9 (1.9–4.1)	8.25 (NE–NE)	4.1 (1.6–4.1)	NE

CI, confidence interval; CR, confirmed response; NE, not estimable; PR, partial response; SD, stable disease.
Data cut-off: April 15, 2022.
[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]Tumor response based on investigator tumor assessments (RECIST version 1.1).
[c]Kaplan-Meier analysis. For crossover patients, calculated from time of crossover to N+F+T.
[d]Clinical benefit defined as confirmed CR or PR or SD for ≥24 weeks (within ± 7-day visit window).

Figure 2. ctDNA samples for central NGS



EOT, end of treatment.

Table 2. Concordance between enrollment assay and central pretreatment ctDNA NGS

Central NGS	Enrollment assay sample type	
Pretreatment ctDNA (centrally assessed), n (%)	FFPE tissue (n=14)	ctDNA (n=10)
HER2 mutation detected	13 (92.8)	8 (80.0)[a]
HER2 mutation not detected	1 (7.1)	2 (20.0)

[a]One of the 8 patients had different *HER2* mutations detected by Tempus xF+ than by the original enrollment assay (Guardant360).

Figure 3. Genomic spectrum of centrally assessed ctDNA at baseline (n=24)

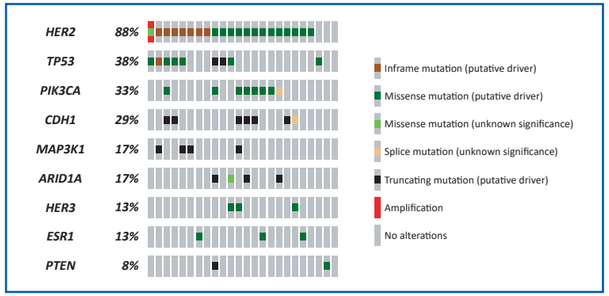
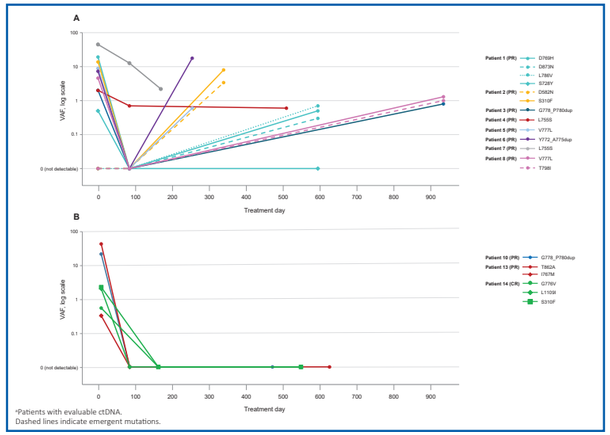


Figure 4. *HER2* mutations: VAF in patients[a] treated with N+F+T. Blood draw and ctDNA sequencing A) at pretreatment, on-treatment, and at time of progression in patients who progressed after treatment and B) at pretreatment, on-treatment, and at last blood draw in patients who remained on treatment

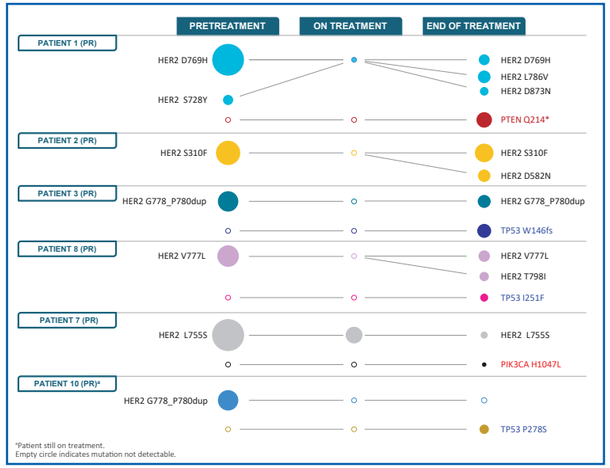


[a]Patients with evaluable ctDNA.
Dashed lines indicate emergent mutations.

HER2 mutations in patients treated with N+F+T

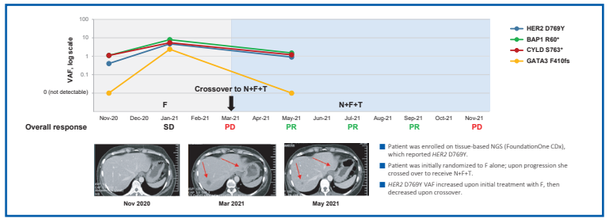
- *HER2* VAFs decreased upon treatment with N+F+T in patients with clinical response, and re-emerged upon progression, along with additional *HER2* mutations, including gatekeepers, sensitive mutations, and variants of unknown significance (Figure 4A).
- *HER2* VAFs decreased upon treatment initiation in patients with clinical response to N+F+T, and remained undetectable while patients remained on treatment (Figure 4B).
- Individual patient mutation profiles are shown in Figure 5 and detail emergence of mutations upon disease progression.

Figure 5. Emergent mutations at progression on N+F+T



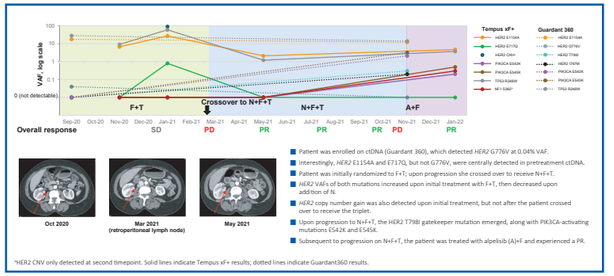
[a]Patient still on treatment.
Empty circle indicates mutation not detectable.

Crossover case study 1: F to N+F+T



- Patient was enrolled on tissue-based NGS (FoundationOne CDx), which reported *HER2* D769Y.
- 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 crossover.

Crossover case study 2: F+T to N+F+T



- Patient was enrolled on ctDNA (Guardant 360), which detected *HER2* G776V at 0.04% VAF.
- Interestingly, *HER2* E1154A and E717Q, but not G776V, were centrally detected in pretreatment ctDNA.
- 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.
- Subsequent to progression on N+F+T, the patient was treated with alpelisib (A)+F and experienced a PR.

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

Conclusions

- *HER2* mutation VAFs in ctDNA from patients with HR+, *HER2*-mutant MBC decrease upon treatment with N+F+T and increase upon progression, consistent with tumor response over time.
- Enrollment *HER2* mutations detected by local clinical assays on either archival primary or metastatic tissue, or liquid biopsy, were 88% overall concordant with centrally assessed ctDNA analysis of pretreatment blood samples.
- The spectrum of genomic alterations was consistent with prior SUMMIT breast cancer cohorts and publicly available datasets. In 12 of the 14 patients who had clinical response to N+F+T and longitudinal ctDNA sequencing, the *HER2* mutation was undetectable in the on-treatment sample; only those two with *L755S* remained detectable on treatment. This observation is consistent with the reported lesser sensitivity of L755S relative to other *HER2* mutations.[1,7,9]
- Mutations that emerged upon progression on N+F+T in patients with initial clinical response included additional *HER2* alterations (gatekeeper mutations, sensitive mutations, and variants of unknown significance) and mutations in *PIK3CA*, *PTEN*, and *TP53*.
- Dual HER2 targeting plus HR targeting (N+F+T), despite deepening and prolonging clinical response compared with N+F alone, did not preclude eventual emergence of additional HER2 genomic events.
- Highlighted case studies of patients initially randomized to F or F+T who then crossed over to receive the triplet, with corresponding ctDNA analysis and imaging results, support the role of N in the efficacy of the triplet regimen.

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