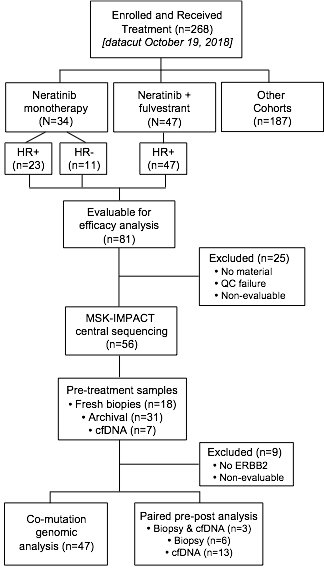
# Supplementary data

A close up of a map

Description automatically generated

**Supplementary Figure S1.** PFS of (A) neratinib plus fulvestrant combination cohort and (B) neratinib monotherapy cohort.

CI, confidence interval; PFS, progression-free survival.



**Supplementary Figure S2.** Overall sequencing CONSORT diagram.Flow diagram of study patients and analyzed biospecimens processed through and selected for central sequencing using MSK-IMPACT and Guardant360.

cfDNA, cell-free DNA; FFPE, formalin-fixed paraffin-embedded; MSK-IMPACT, Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets; QC, quality control.

**A screenshot of a cell phone

Description automatically generated**

**Supplementary Figure S3.** Comprehensive OncoPrint of nine patients with paired pre- and post-treatment tissue sequencing showing acquired alterations in the post-treatment tumor.

**Supplementary Table S1. Patient disposition**

|  |  |  |
| --- | --- | --- |
| **Patients, *n* (%)** | **Neratinib monotherapy**  **(*n*=34)** | **Neratinib + fulvestrant on Day 1**  **(*n*=47)** |
| **Patients enrolled** | 34 (100) | 47 (100) |
| **Enrolled and received study drug** | 34 (100) | 47 (100) |
| **Patients received study drug and continuing treatment** | 0 | 8 (17.0) |
| **Patients discontinued treatment** | 34 (100) | 38 (80.9) |
| Death | 0 | 1 (2.1) |
| Disease progression | 32 (94.1) | 34 (72.3) |
| Adverse event | 0 | 1 (2.1) |
| Withdrawal of consent | 1 (2.9) | 0 |
| Other | 1 (2.9) | 2 (4.3) |
| **Patients ended study** | 25 (73.5) | 21 (44.7) |
| Death | 23 (67.6) | 17 (36.2) |
| Withdrawal of consent | 1 (2.9) | 2 (4.3) |
| Lost to follow-up  Other | 1 (2.9)  0 | 1 (2.1)  1 (2.1) |

**Supplementary Table S2. *HER2* mutational characteristics**

|  |  |  |  |
| --- | --- | --- | --- |
| **Domain** | **No. of patients (%)** | | |
| **All patients (*n*=81)** | **Neratinib monotherapy (*n*=34)** | **Neratinib + fulvestrant combination therapy (*n*=47)** |
| PKD hotspot | 48 (59.3) | 22 (64.7) | 26 (55.3) |
| Exon 20 insertion hotspot | 15 (18.5) | 6 (17.6) | 9 (19.1) |
| S310 hotspot | 10 (12.3) | 3 (8.8) | 7 (14.9) |
| Other hotspot | 2 (2.5) | 0 | 2 (4.3) |
| Other non-hotspot | 6 (7.4) | 3 (8.8) | 3 (6.4) |

PKD, protein kinase domain.

**Supplementary Table S3. Treatment efficacy and impact of prior fulvestrant and CDK4/6 inhibitor-containing therapy on treatment outcome**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Neratinib monotherapya** | | **Neratinib + fulvestrant** | |
| **Prior fulvestrant** | **Yes** | **No** | **Yes** | **No** |
| **All patients (intent to treat)b** | (*n*=12) | (*n*=11) | (*n*=25) | (*n*=22) |
| **Confirmed overall objective responsec, *n* (%)**  Complete response  Partial response  Overall objective response rate (95% CI) | 4 (33.3) 2 (16.7)  2 (16.7)  33.3 (9.9–65.1) | 0  0  0  0 (0–28.5) | 4 (16.0) 0 4 (16.0) 16.0 (4.5–36.1) | 10 (45.5) 4 (18.2)  6 (27.3)  45.5 (24.4–67.8) |
| **Clinical benefit rated, % (95% CI)** | 50.0 (21.1–78.9) | 9.1 (0.2–41.3) | 36.0 (18.0–57.5) | 59.1 (36.4–79.3) |
| **Time to event (months), median (95% CI)**  Progression-free survival  Duration of response | 4.8 (1.7, 9.0)  6.5 (3.7–NA) | 3.5 (1.3–3.7)  NA | 3.7 (3.5–6.9)  16.6 (9.2–16.6) | 8.2 (3.8–14.5)  7.4 (3.9–NA) |
| **Prior CDK4/6 inhibitor** | **Yes** | **No** | **Yes** | **No** |
| **All patients (intent to treat)b** | (*n*=2) | (*n*=21) | (*n*=20) | (*n*=27) |
| **Confirmed overall objective responsec, *n* (%)**  Complete response  Partial response  Overall objective response rate (95% CI) | 0  0  0  0 (0–84.2) | 4 (19.0)  2 (9.5)  2 (9.5)  19.0 (5.4–41.9) | 6 (30.0) 1 (5.0) 5 (25.0) 30.0 (11.9–54.3) | 8 (29.6) 3 (11.1)  5 (18.5)  29.6 (13.8–50.2) |
| **Clinical benefit rated, % (95% CI)** | 0 (0–84.2) | 33.3 (14.6–57.0) | 40.0 (19.1–63.9) | 51.9 (31.9–71.3) |
| **Time to event (months), median (95% CI)**  Progression-free survival  Duration of response | 2.8 (1.8–3.7)  NA | 3.6 (1.8–5.4)  6.5 (3.7–NA) | 4.1 (1.9–10.9)  NA (4.5–NA) | 6.9 (3.6–14.5)  9.0 (3.9–16.6) |

CDK, cyclin-dependent kinase; CI, confidence interval; NA, Not available; RECIST, Response Evaluation Criteria in Solid Tumors.

aEstrogen receptor-positive patients only. bResponse is based on investigator-assessment per RECIST (version 1.1), in patients with measurable disease, or positron-emission tomography response criteria in patients without measurable disease. cConfirmed no less than 4 weeks after the criteria for response were initially met. dClinical benefit defined as confirmed best overall response of complete response, partial response of any duration, or stable disease lasting for ≥24 weeks.

**Supplementary Table S4. Adverse eventsa**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Event** | **Neratinib monotherapy (*n*=34)** | | **Neratinib + fulvestrant (*n*=47)** | | **All patients (*n*=81)** | |
| **Any grade** | **Grade ≥3** | **Any grade** | **Grade ≥3** | **Any grade** | **Grade ≥3** |
| Any adverse event | 33 (97.1) | 16 (47.1) | 47 (100) | 23 (48.9) | 80 (98.8) | 39 (48.1) |
| Diarrhea | 26 (76.5) | 9 (26.5) | 40 (85.1) | 11 (23.4) | 66 (81.5) | 20 (24.7) |
| Fatigue | 16 (47.1) | 0 | 12 (25.5) | 0 | 28 (34.6) | 0 |
| Nausea | 15 (44.1) | 0 | 21 (44.7) | 0 | 36 (44.4) | 0 |
| Constipation | 14 (41.2) | 0 | 15 (31.9) | 0 | 29 (35.8) | 0 |
| Vomiting | 13 (38.2) | 1 (2.9) | 10 (21.3) | 1 (2.1) | 23 (28.4) | 2 (2.5) |
| Abdominal pain | 8 (23.5) | 1 (2.9) | 8 (17.0) | 0 | 16 (19.8) | 1 (1.2) |
| Decreased appetite | 8 (23.5) | 0 | 13 (27.7) | 0 | 21 (25.9) | 0 |
| AST increased | 7 (20.6) | 3 (8.8) | 3 (6.4) | 1 (2.1) | 10 (12.3) | 4 (4.9) |
| Arthralgia | 6 (17.6) | 0 | 6 (12.8) | 0 | 12 (14.8) | 0 |
| Pyrexia | 6 (17.6) | 0 | 4 (8.5) | 0 | 10 (12.3) | 0 |
| Anemia | 5 (14.7) | 2 (5.9) | 6 (12.8) | 1 (2.1) | 11 (13.6) | 3 (3.7) |
| Dyspnea | 5 (14.7) | 2 (5.9) | 6 (12.8) | 1 (2.1) | 11 (13.6) | 3 (3.7) |
| Headache | 5 (14.7) | 0 | 6 (12.8) | 0 | 11 (13.6) | 0 |
| ALT increased | 4 (11.8) | 1 (2.9) | 2 (4.3) | 0 | 6 (7.4) | 1 (1.2) |
| Dehydration | 4 (11.8) | 2 (5.9) | 2 (4.3) | 0 | 6 (7.4) | 2 (2.5) |
| Pruritus | 4 (11.8) | 0 | 4 (8.5) | 0 | 8 (9.9) | 0 |
| Rash | 4 (11.8) | 0 | 7 (14.9) | 0 | 11 (13.6) | 0 |
| Abdominal distension | 4 (11.8) | 0 | 2 (4.3) | 0 | 6 (7.4) | 0 |
| Dry skin | 3 (8.8) | 0 | 9 (19.1) | 0 | 12 (14.8) | 0 |
| Back pain | 3 (8.8) | 1 (2.9) | 8 (17.0) | 0 | 11 (13.6) | 1 (1.2) |
| Insomnia | 2 (5.9) | 0 | 5 (10.6) | 0 | 7 (8.6) | 0 |
| Peripheral edema | 1 (2.9) | 0 | 7 (14.9) | 0 | 8 (9.9) | 0 |
| Weight decreased | 1 (2.9) | 0 | 5 (10.6) | 0 | 6 (7.4) | 0 |
| Hot flash | 0 | 0 | 5 (10.6) | 0 | 5 (6.2) | 0 |

aRegardless of attribution, occurring in ≥10% of patients.

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

**Supplementary Table S5. Outcomes in patients with concurrent *HER2* and *HER3* mutations**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Patient** | **BOR** | **PFS (weeks)** | ***ERBB2* domain** | **Mutation** | |
| ***ERBB2*** | ***ERBB3*** |
| 3 | PD | 3.6 | Extracellular hotspota | S310F/D582N | E332K |
| 12 | PD | 7.6 | Kinase domain, hotspot | L755S | E928G |
| 20 | SD | 11.2 | Kinase domain, non-hotspot | L841V | K329I |
| 24 | SD | 14.0 | Kinase domain, hotspot | L755S | E928G |
| 36 | uPR | 14.0 | Kinase domain, hotspot | L869R | E928G |
| 39 | uPRb | 15.6 | Kinase domain, hotspot | L869R | E928G |
| 56 | uPR | 14.0 | Juxtamembrane, hotspot | V697L | K329E |
| 79 (ongoing) | cCR | 52.0 | Extracellular hotspot | S310F | K329E/S846I |

BOR, best overall response; c, confirmed; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; Tx, treatment; u, unconfirmed.

aRefers to S310F mutation; D582N occurs in the extracellular domain and is a non-hotspot mutation. bAccording to positron-emission tomography response.

**Supplementary Table S6. Acquired *ERBB2* sequencing results from paired tumor and cfDNA analysis**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **BOR**  **(Tx duration, wks)** | **Pre-treatment** | | | **Post-treatment** | | |
| **ERBB2** | **Tissue AF** | **Plasma AF** | **ERBB2** | **Plasma AF** | **Tissue AF** |
| 47 | cPR (32) | G778\_P780dup | 0.51 | 0.04 | G778\_P780dup  I767M  Amplification  S310Y | 0.41  0.0034  Detected  ND | 0.56  ND  Detected  0.44 |
| 36 | uPR (64.1) | L869R | 0.68 | 0.06 | L869R  S310Y  D769Y  L755S  T798I | 0.14  0.0015  0.0009  0.0022  0.0096 | 0.44  ND  ND  ND  ND |
| 56 | uPR (16.0) | V697L | 0.14 | 0.018 | V697L  Amplification | 0.0058  ND | 0.74  Detected |
| 45 | SD (30.1) | L755S  L755P | 0.29  ND | 0.045  0.0086 | L755S  L755P  T862A  S310F | 0.24  0.14  0.0017  0.0022 | NA |
| 17 | PD (9.0) | L755S | 0.09 | 0.06 | L755S  D769H  K1171N  D1016Y  D1089Y  D962H | 0.14  0.0017  0.002  0.0029  0.0039  0.0042 | NA |
| 59 | cPR (88.0) | V777L | 0.21 | 0.015 | V777L  T798I | 0.02  0.0057 | NA |
| 57 | SD (88.6) | G776V | 0.05 | 0.053 | G776V  I767M | 0.11  0.0018 | NA |
| 60 | cCR (80.0) | S310F | 0.10 | NA | S310F  L785F | NA | 0.11  0.10 |

AF, allele frequency; BOR, best overall response; c, confirmed; cfDNA, cell-free DNA; CR, complete response; NA, not available; ND, not detected; PD, progressive disease; PR, partial response; SD, stable disease; Tx, treatment; u, unconfirmed.

**Supplementary Table S7. PET Response Criteria**

|  |  |
| --- | --- |
| **Response category** | Based on sum of SUVmax from 1 to 5 target lesions. Each target lesion with initial SUVmax of >1.5 × normal liver background SUVmax |
| **Complete metabolic response (CMR)** | Reduction of SUVmax of all target lesions to less than normal liver background SUVmax (for non-brain lesions) or less than normal brain background SUVmax (for brain lesions)  AND  The reduction of all other FDG-avid lesions consistent with disease to less than normal liver background SUVmax |
| **Partial metabolic response (PMR)** | Sum of SUVmax of all target lesions is decreased by ≥30% compared to baseline sum of SUVmax of all target lesions  AND  No new lesions |
| **Stable metabolic disease (SMD)** | Not satisfying the criteria for CMR, PMR, PMD, or NE |
| **Progressive metabolic disease (PMD)** | Sum of SUVmax of all target lesions is increased by ≥30%  OR  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, 18F-fluorodeoxyglucose positron-emission tomography; SUVmax, maximum standardized uptake value.