Memorial Sloan Kettering Cancer Center

Paired tumor and cfDNA in patients with HER2-mutant solid tumors treated with neratinib reveals convergence of multiple on-target resistance mechanisms: Results from the SUMMIT "Basket" Trial

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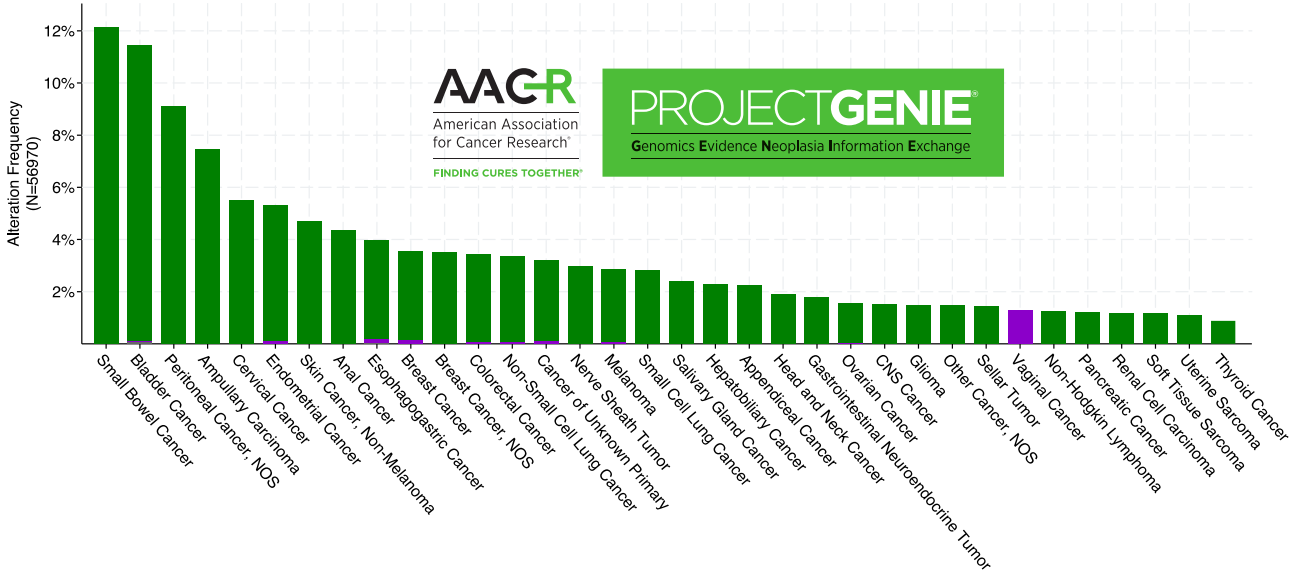
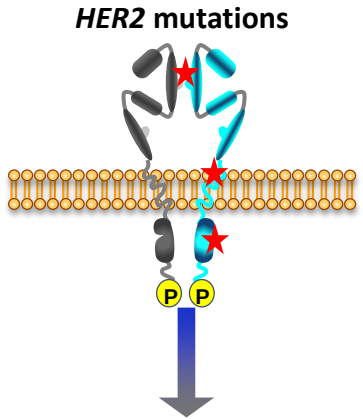
HER2 (*ERBB2*) mutations

- Somatic HER2 mutations are seen at relatively low frequencies across multiple tumor types
- Unlike many oncogenic drivers, mutations in HER2 occur across multiple domains of the protein, specifically in the extracellular, transmembrane, and kinase domain, resulting in constitutive kinase signaling, oncogenic transformation and enhanced tumor growth in preclinical models



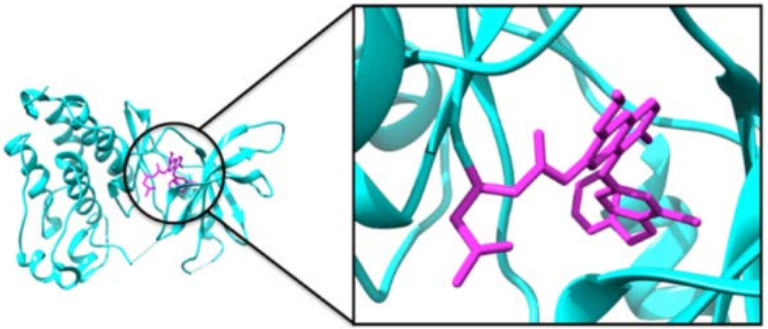


Activation of downstream signal transduction pathways and tumor growth survival

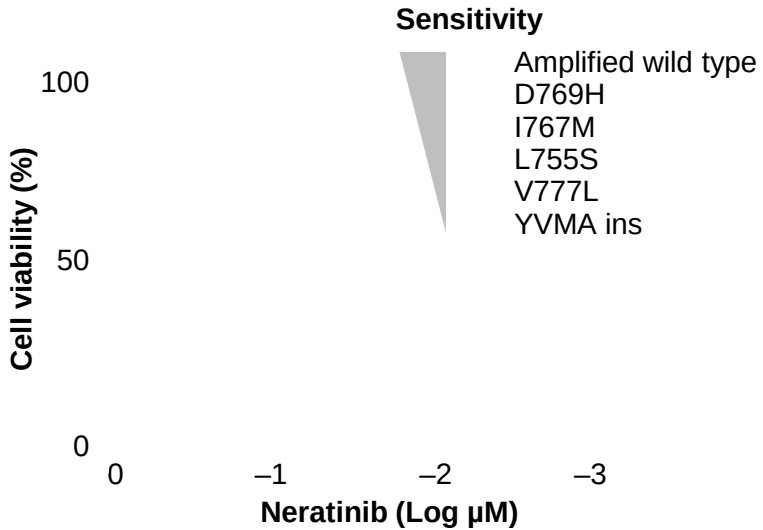
GENIE Cohort v.5.0

Neratinib (HKI-272; PB272; NERLYNX®)

- Neratinib is an oral, irreversible pan-HER tyrosine kinase inhibitor and suppresses intracellular signaling, cell proliferation and colony formation in HER2-mutant tumor cell lines *in vitro*[1,2]
- In preclinical models, HER2-mutant alleles activate to different degrees and have differential sensitivity both within and across HER tyrosine kinase inhibitors



Covalent binding to conserved cysteine residues in the kinase active binding site of EGFR, HER2 and HER4[3]

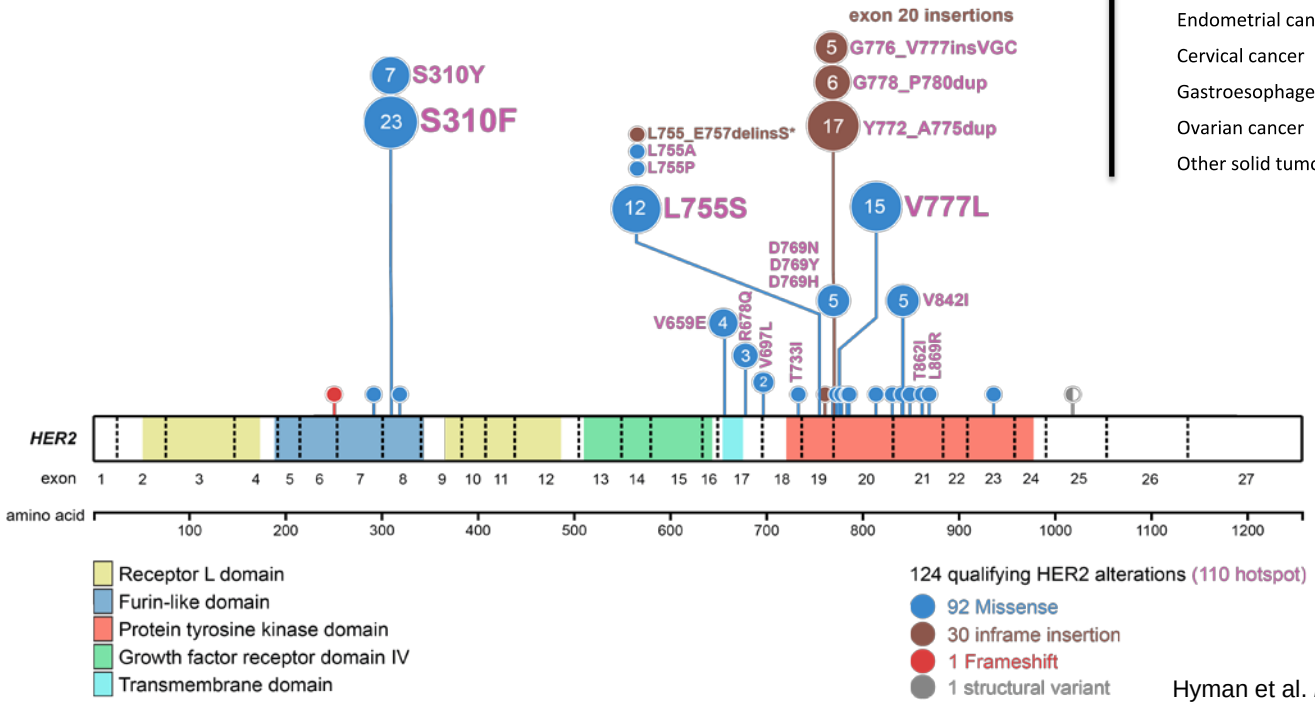


[1]Bose et al. Cancer Discov 2013;3:224–37.
[2]Carmona et al. Cancer Res 2016:76(14 Suppl);abst 298
[3]Wissner & Mansour. Arch Pharm Chem Life Sci 2008;341:465–477

SUMMIT neratinib ‘basket’ study

- 125 patients enrolled in the HER2 cohort
- 31 unique mutations, 87% hotspot mutations

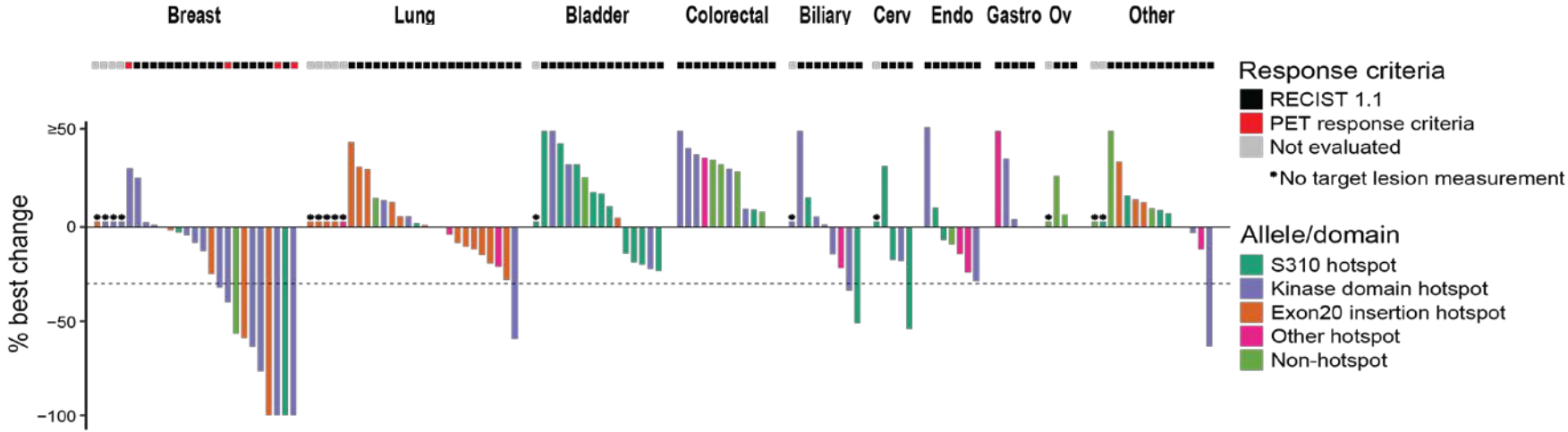
HER2-mutation positive	
Lung cancer	26 (18.4)
Breast cancer	25 (17.7)
Bladder/urinary tract cancer	16 (11.3)
Colorectal cancer	12 (8.5)
Biliary tract cancer	9 (6.4)
Endometrial cancer	7 (5.0)
Cervical cancer	5 (3.5)
Gastroesophageal cancer	5 (3.5)
Ovarian cancer	4 (2.8)
Other solid tumors (NOS)	15 (10.6)



Hyman et al. *Nature*. 2018 Feb 8; 554(7691): 189-194.

Efficacy in HER2-mutant patients by tumor type/allele

- Neratinib activity was influenced by both tumor lineage as well as mutation type
- Greatest degree of response was observed in breast cancer



ORR: 32%

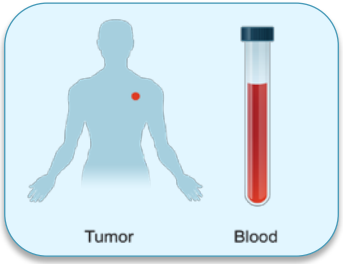
Breast Combination cohort:
Neratinib with fulvestrant in ER+ MBC patients

Hyman et al. *Nature*. 2018 Feb 8; 554(7691): 189-194.

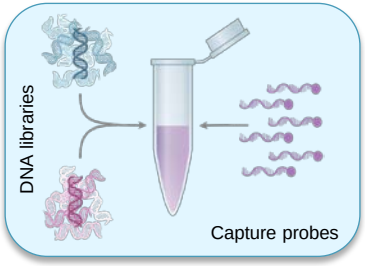
MSK-IMPACT for tissue sequencing

*Deep coverage, targeted sequencing of **468 genes** to guide treatment*

1. Patient Consent

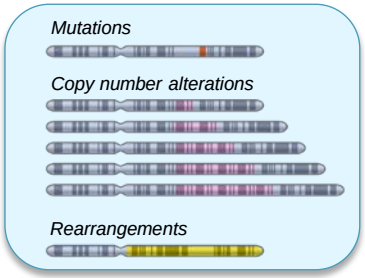


2. Sample Accessioning



3. Sample Preparation

4. Sequencing



5. Bioinformatics Analysis

6. Case Review and Sign Out

<u>Cancer Gene Exons (468 genes)</u>:
- actionable mutations
- targets of investigational agents
- frequently mutated in cancer
- cancer susceptibility genes

<u>Cancer Gene Introns (20 genes)</u>:
- recurrent rearrangements

<u>Noncoding Regions</u>
- TERT promoter
- microsatellites
- >1000 common SNPs

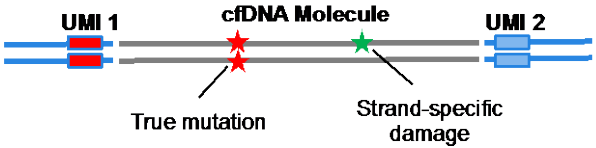
Target Territory = 1.52 Mb
Average Coverage = 720x

Zehir, Benayed *et al.*, *Nature Medicine*, 2017

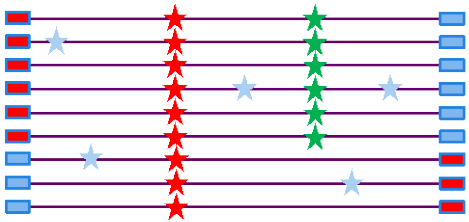
MSK-ACCESS for cfDNA sequencing

*Ultra-deep coverage, targeted sequencing of **129 genes***

'Duplex' Unique Molecular Indexing



Amplify and Sequence ~20,000X



Error-Free Consensus

Leveraged experience from sequencing 25,000 tumors with MSK-IMPACT

Selected exons of 129 genes for mutation detection
- OncoKB Level 1-4
- Hotspot sites
- High rates of mutations
- Protein kinase domains
- Tumor suppressor genes

Microsatellite regions

SNPs for zygosity and copy number of 13 genes

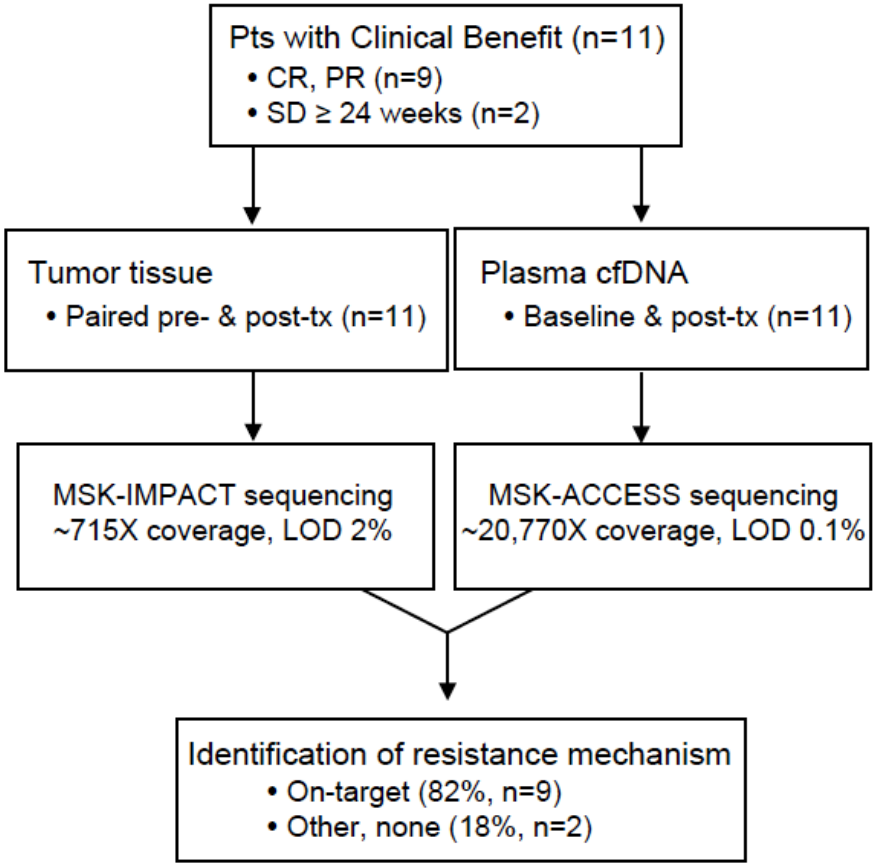
Common SNPs for genome-wide copy number & QC

Introns for structural variants of 10 genes

Clonal hematopoiesis genes

Submitted to NYS Department of Health for clinical use approval

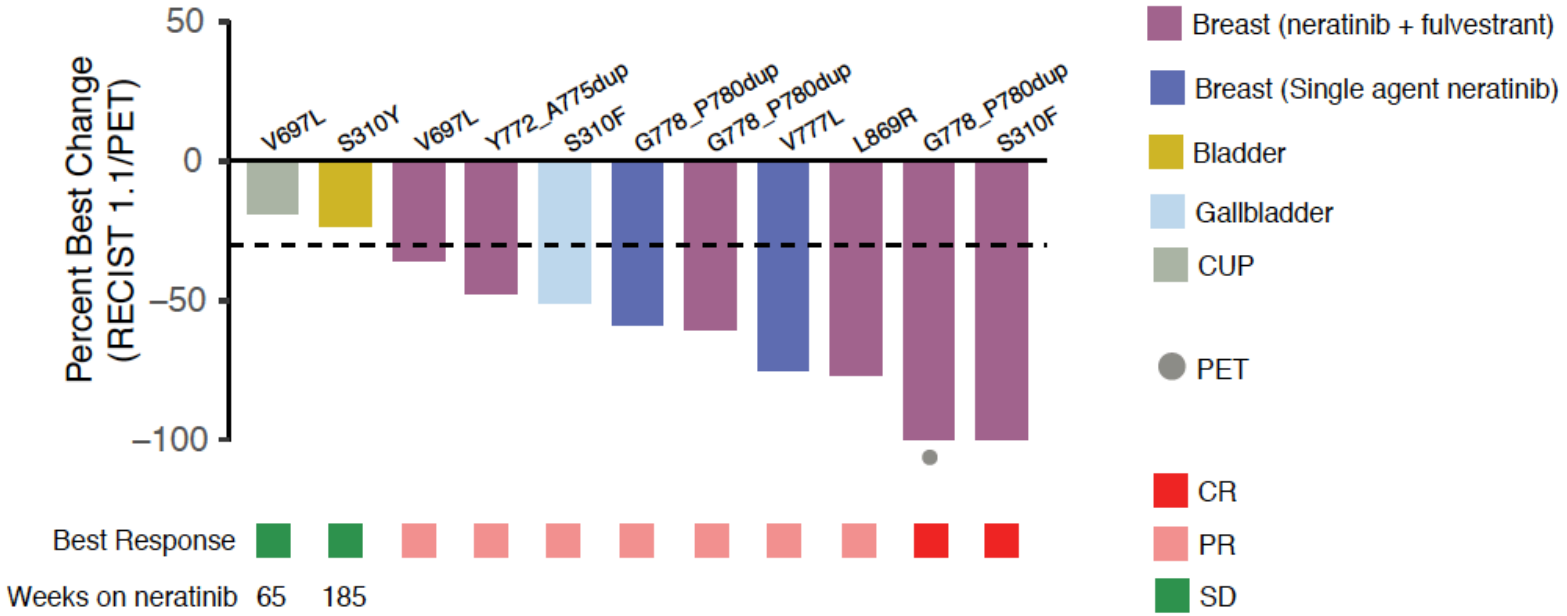
Acquired Resistance Patient Cohort Description



LOD: Limit of Detection

Clinical Response by Tumor Type and Allele

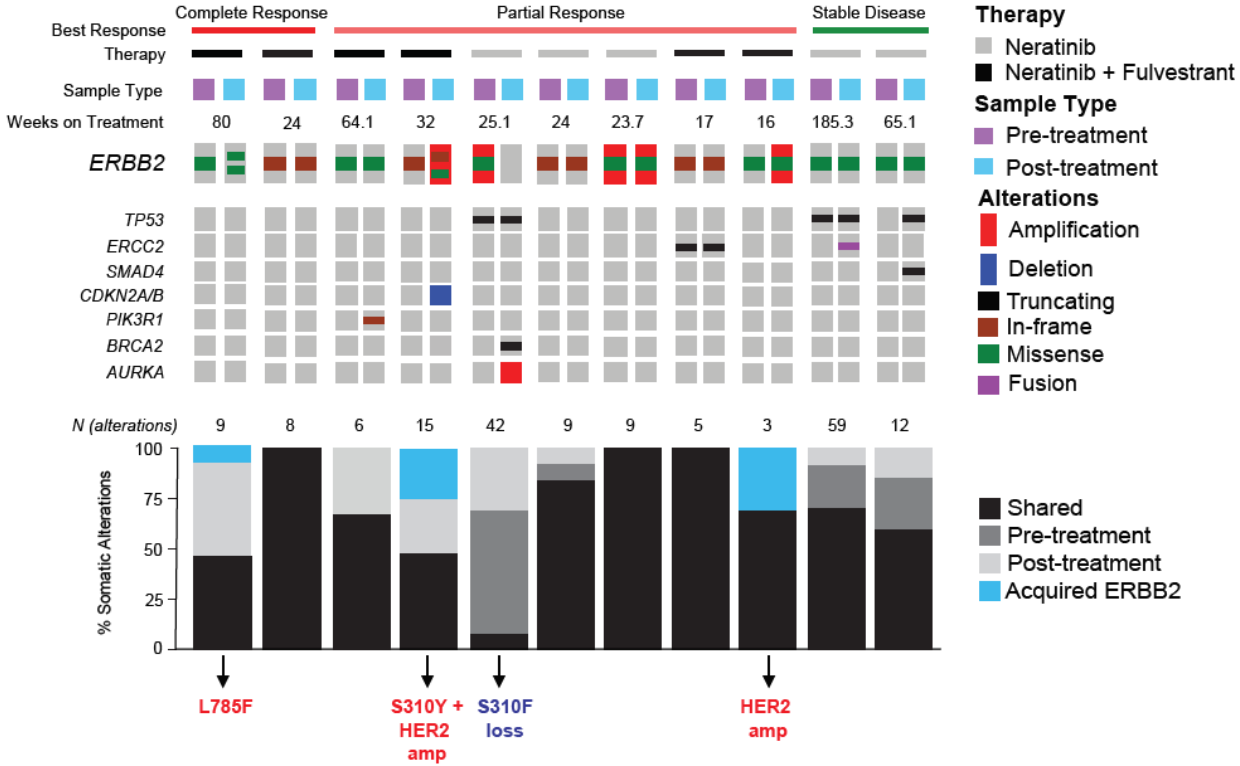
- 11 patients with significant clinical response (RECIST 1.1 or PET)
- All enrolling HER2 mutations were clonal



CUP: Cancer of unknown primary

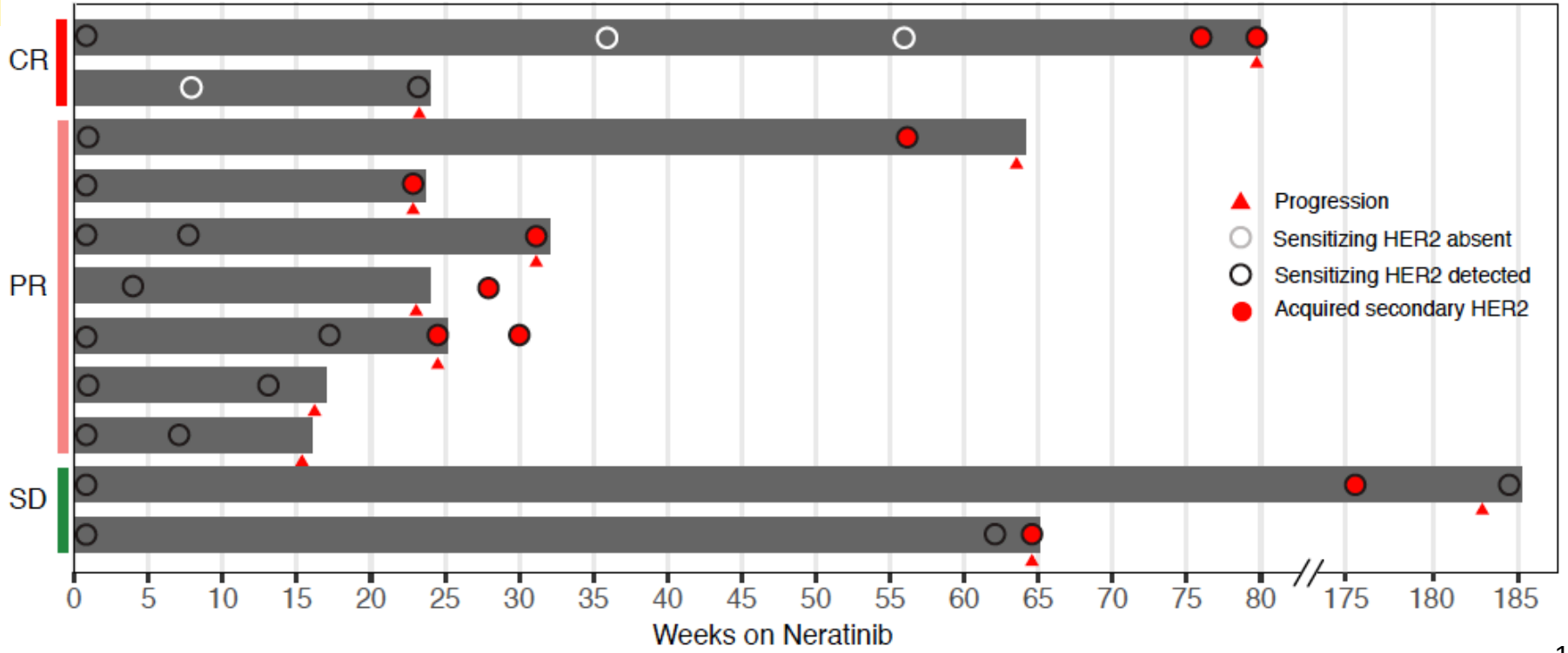
Acquired HER2 resistance in tissue

- Pretreatment HER2 mutation retained in 10/11 tissues at progression
- 73% (8/11) acquired at least one alteration at progression
  - 38% (3/8) acquired a secondary HER2 alteration



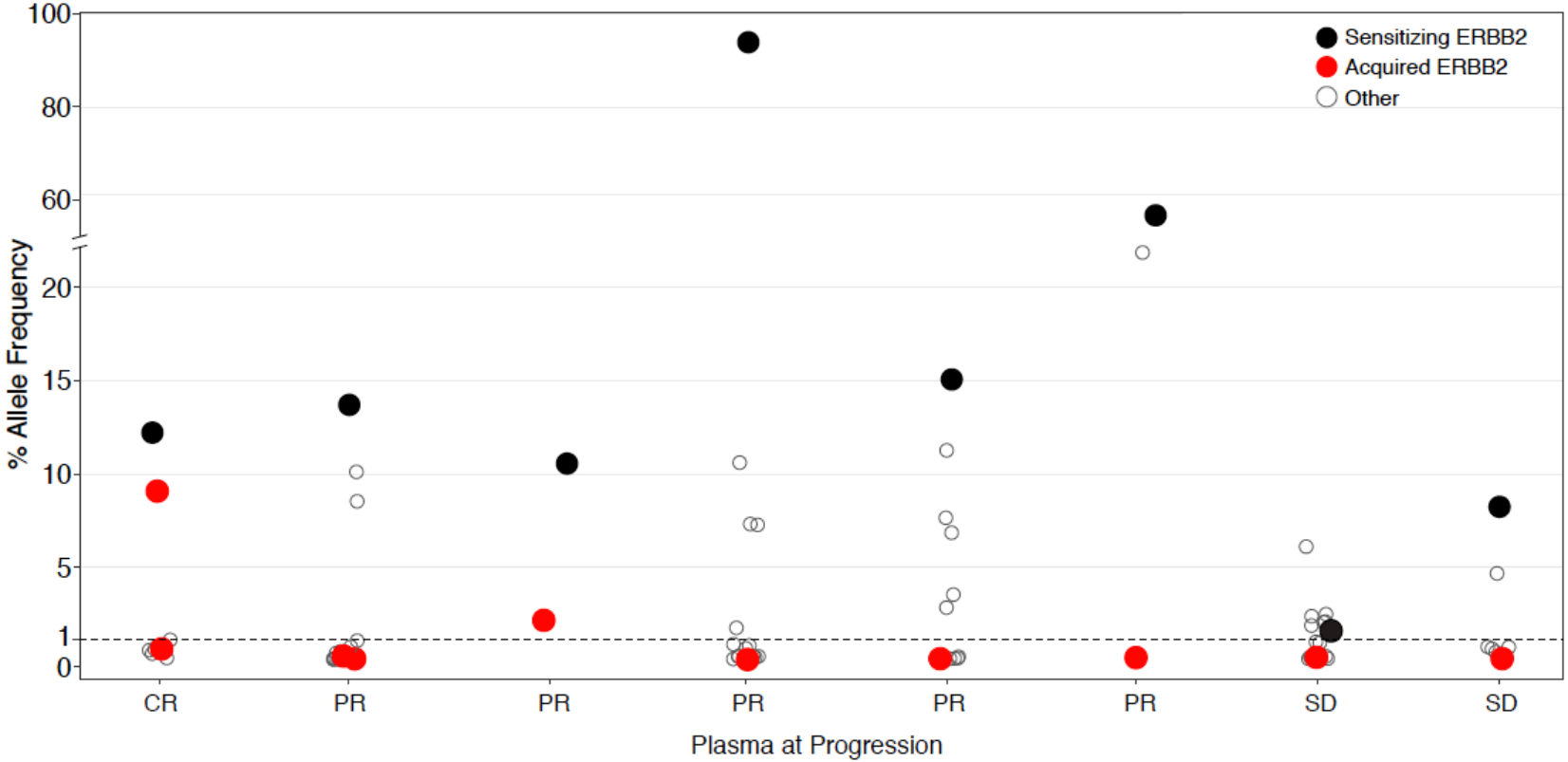
Acquired HER2 resistance in plasma cfDNA

- 73% (8/11) acquired a secondary HER2 alteration post-neratinib in cfDNA



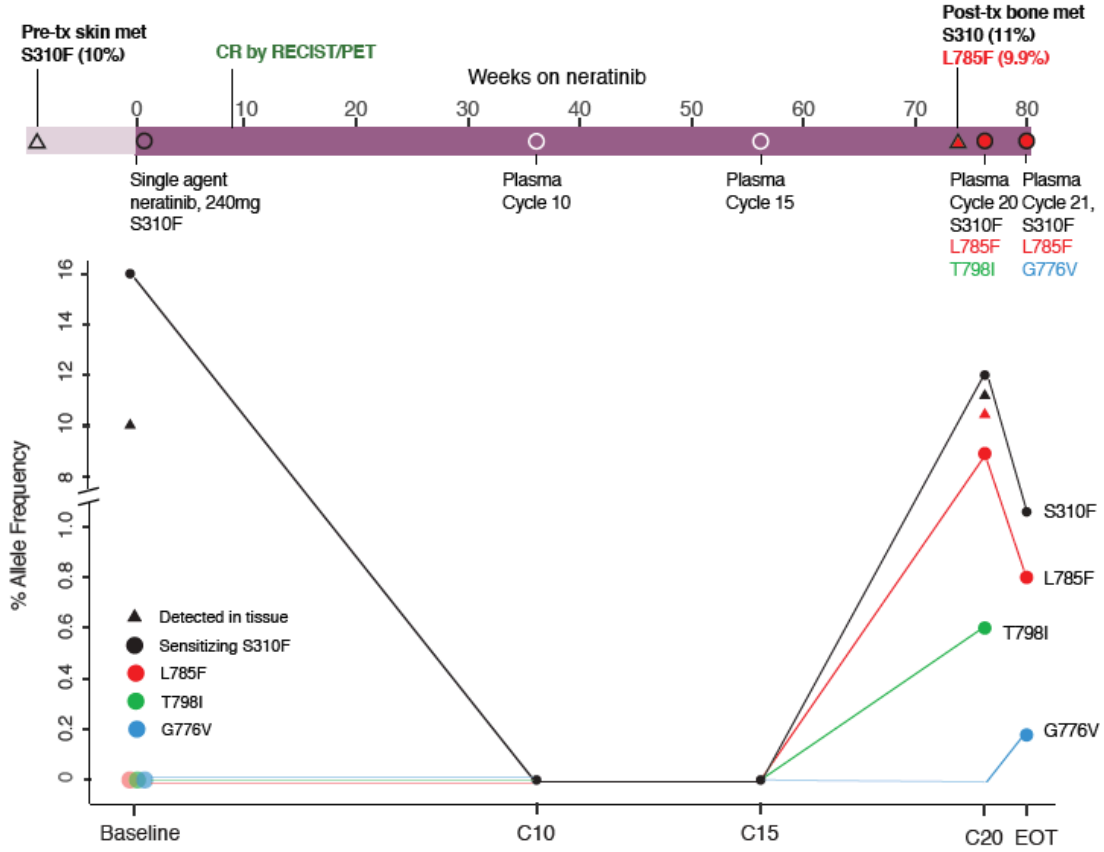
Acquired HER2 mutations in cfDNA seem subclonal

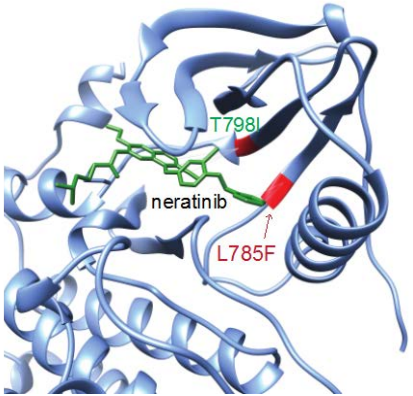
- Subclonal HER2 mutations acquired near, at, or post progression (range 0.1% - 9.1%)



Acquisition of Gatekeeper Mutations in an ER+ Invasive Lobular Breast Cancer Patient

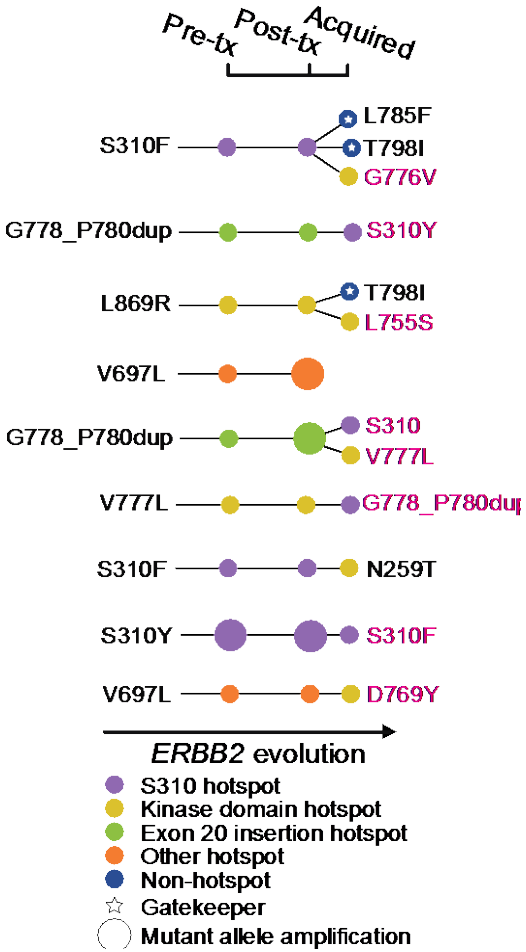
83 year-old female
ER+/PR-/HER2-
CR, 80 weeks





Overall ERBB2 evolution

- On-target mutations identified in 82% (9/11)
  - 78% activating (7/9)
  - 22% gatekeeper (2/9)
  - 11% non-hotspot (1/9) with polyclonal resistance



Conclusions

- In patients with clinical benefit on neratinib, a potential on-target resistance mechanism was identified in 82% (9 of 11 patients)
  - Gatekeeper mutations were acquired in two patients treated on combination fulvestrant therapy with prior HER2-targeted therapies
- HER2 mutations lead to oncogene addiction in solid tumors and HER2 signaling may select for acquisition of additional activating events
- Tumor sequencing in combination with plasma cfDNA sequencing can be utilized to provide insight into intra-tumor and subclonal heterogeneity
  - Additional tissue and longitudinal plasma sampling is essential in providing a more comprehensive overview of molecular response and resistance mechanisms

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