Oncogenic activity of recurrent ERBB4 mutations and their sensitivity to neratinib

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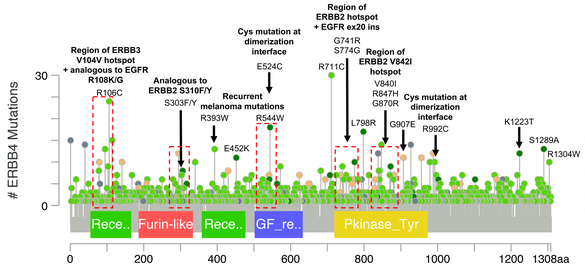
Introduction

- Hundreds of somatic ERBB4 mutations have been reported in various cancer tissues but their clinical significance is poorly understood.
- Several oncogenic ERBB4 mutations have been described but the most recurrent mutations have remained uncharacterized.
- Understanding the functional consequences of ERBB4 mutations is critical to assess the relevance of targeting ERBB4 in cancer.
- Clinically approved pan-ERBB inhibitors, such as neratinib, potently inhibit ERBB4.

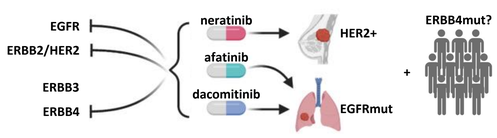


ERBB4 mutation	Number of mutations in cBioPortal
R106C/H/P	24
S303F/Y	7
R393W/Q	13
E452K/D	11
R524C/H	6
R544W/Q	18
R711C/G/H/L	30
G741E/R	9
S774G/R/C/N	10
L798R/P/H/I	17
V840I/L/S	8
R847H/C/L	14
G870R/E	7
G907E/A	5
R992C/H/S	10
K1223T/R/N/M	12
S1289A/F	13
R1304W/L/Q	9

Figure 1. ERBB4 mutations reported in cBioPortal (curated non-redundant studies) as of August 2022. Regions of interest (red boxes) demonstrate 1) analogy to activating mutations described for other oncogenic ERBB family members and/or 2) location at dimerization interface suggesting functional relevance. The 18 recurrent missense mutations chosen for analyses are indicated as well as the number of patients with these mutations in cBioPortal.

Hypothesis

Recurrent ERBB4 mutations could be oncogenic and potentially targetable with clinically approved pan-ERBB inhibitors.



Screen of the transforming potential of recurrent ERBB4 mutations

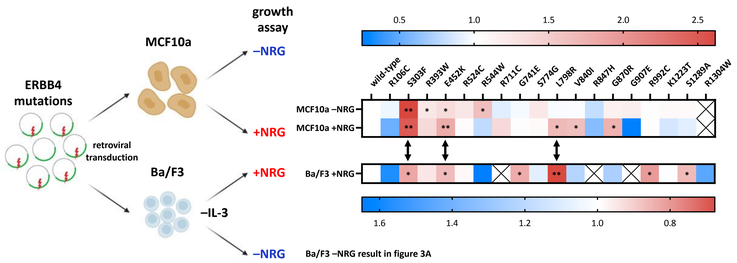
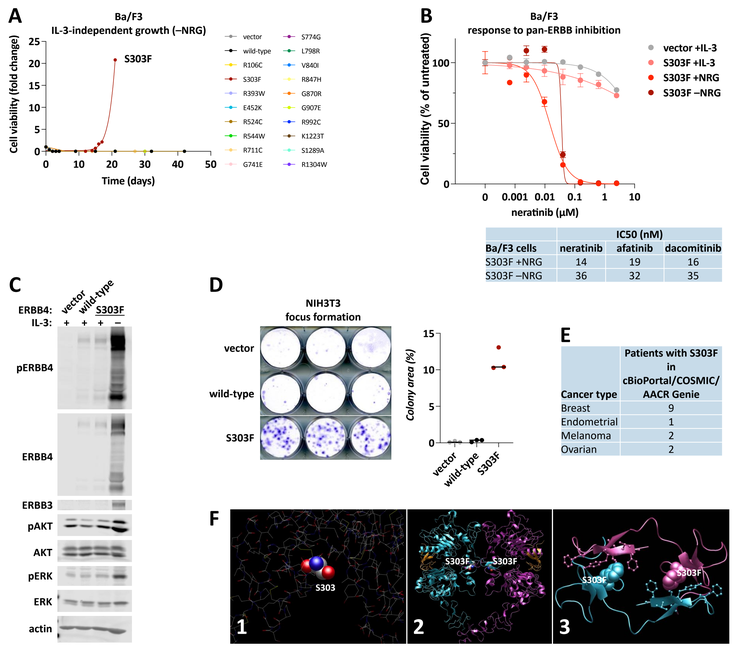


Figure 2. Schematic and results of the screen. Heatmaps represent MCF10a human mammary epithelial cell viability fold change and mouse lymphoid Ba/F3 cell doubling time fold change to wild-type ERBB4 expressing cells in the presence or absence of ERBB4 ligand neuregulin-1 (NRG). *, q<0.05; **, q<0.01 (n=4-28, Kruskal-Wallis test, FDR-corrected). Arrows indicate mutants with significant transforming potential in both cell lines.

ERBB4 S303F, analogous to ERBB2 S310F, is strongly oncogenic



Ba/F3 cells	IC50 (nM) neratinib	afatinib	dacomitinib
S303F +NRG	14	19	16
S303F –NRG	36	32	35

Cancer type	Patients with S303F in cBioPortal/COSMIC/ AACR Genie
Breast	9
Endometrial	1
Melanoma	2
Ovarian	2

Figure 3. A) IL-3- and ligand-independent growth of Ba/F3 cells expressing mutant or wild-type ERBB4. **B)** Ba/F3 cell response to pan-ERBB-inhibition and table of IC50 values. **C)** ERBB4 activity and downstream signaling in Ba/F3 cells, analyzed by western. IL-3- and ligand-independently growing cells upregulate ERBB4 and ERBB3 expression. **D)** Focus formation of NIH3T3 cells expressing ERBB4 wild-type or S303F and quantification of crystal violet stained foci using ColonyArea ImageJ plug-in. **E)** Table of the number of patients with ERBB4 S303F mutation (in cBioPortal, COSMIC and AACR Genie as of January 2022) by cancer type. **F)** Structural analysis of S303F: Serine 303 (1) substitution by aromatic phenylalanine (2) is predicted to stabilize interactions with the hydrophobic side chains (ball-and-stick) of the dimerization partner (magenta) (3), leading to increased receptor activity and downstream signaling.

ERBB4 L798R modulates pan-ERBB inhibitor sensitivity

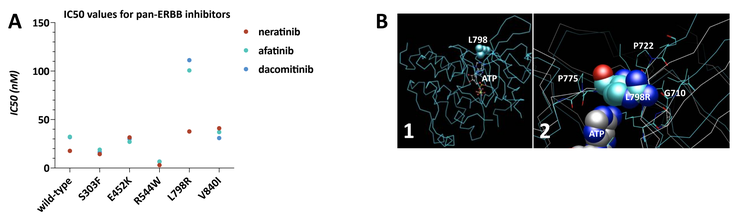


Figure 4. A) Scatter plot of pan-ERBB inhibitor IC50 values for Ba/F3 cells expressing wild-type or mutant ERBB4. Cells were cultured in the presence of 10ng/ml NRG. **B)** Structural analysis of L798R: L798 is located in the ATP-binding pocket of ERBB4 kinase domain (1). ERBB4 L798R interactions with the indicated residues are predicted to stabilize the active state (cyan versus white inactive state) of the receptor (2).

Patient cases

Table 1. Patients whose tumors harbored ERBB4 mutations, enrolled in PUMA-NER-5201, the SUMMIT study (NCT01953926) and were treated with neratinib as a single agent*. Arrows indicate ERBB4 mutations functionally characterized in this study.

ERBB4 mutation	Co-altered genes	Cancer type	Prior lines of therapy	Best response	PFS (months)	OS (months)
N465K	PIK3CA	Ovarian clear cell carcinoma	4	PD	1.12	2.2
→ V840I	TP53, HER2 amp, CCNE1 amp	Peri-ampullary adenocarcinoma	1	PD	1.74	Not evaluable (withdrew consent)
→ R544W	TP53	Rectal adenocarcinoma	6	PD	1.71	14.3

*Neratinib 240mg/day

Conclusions

- Recurrent ERBB4 mutations have transforming potential.
- S303F is a novel strongly oncogenic ERBB4 mutation and its mechanism of activation resembles that of its analogue, ERBB2 S310F[1].
- S303F is mostly detected in breast cancer patients.
- L798R decreases sensitivity to afatinib and dacomitinib but not to neratinib - partly similarly to mutations in analogous EGFR residue L792[2].
- Future clinical studies of neratinib for ERBB4-mutant cancers may consider enrolling only patients with strongly oncogenic ERBB4 mutations and/or combining neratinib with additional targeted agents or chemotherapies.

References:
[1]Diwanji et al. Nature 2021
[2]Kobayashi et al. Molecular Cancer Therapeutics 2017

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