Breast cancers with heterogeneous *HER2* amplification show a diverse distribution of 'driver' and 'passenger' somatic mutations and copy number variations

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Background

Invasive breast cancers with *HER2* gene amplification are associated with poor outcome. Heterogeneous *HER2* amplification has been observed in up to 41% of breast cancers, depending upon its definition. Carcinogenesis is driven by intra-tumour heterogeneity. Molecular diversity enables cancer cells to circumvent specific targeted treatment.

In this study, we compared the genetic differences between admixed HER2-positive and HER2-negative breast cancer components. This in-depth analysis investigated the heterogeneity in their somatic mutational landscape.

Materials & Methods

Formalin-fixed, paraffin-embedded tissue samples from **ten breast cancer patients** were collected at the Erasmus Medical Center Cancer Institute (Rotterdam, The Netherlands).

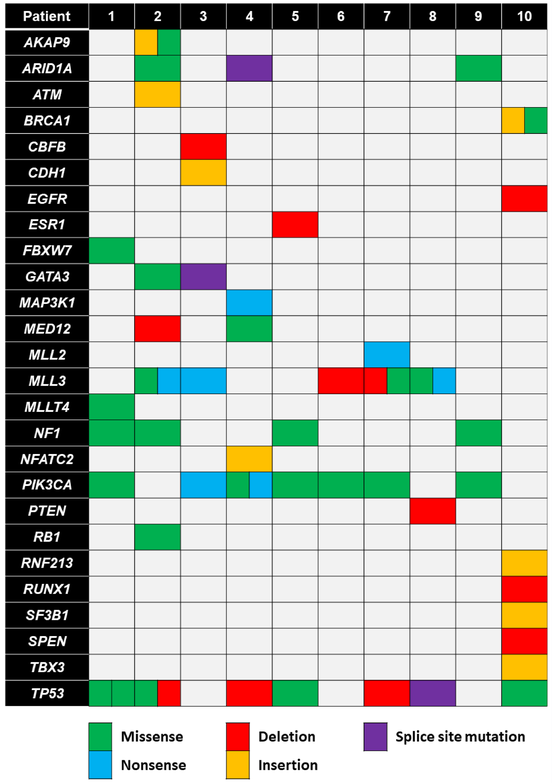
Immunohistochemistry for oestrogen receptor, progesterone receptor, HER2 and p63 was performed. Each carcinoma contained **at least one HER2-negative and at least one HER2-positive component**, as confirmed by silver *in situ* hybridization analysis (**SISH**).

All samples were micro-dissected. Each component was subjected to **targeted next-generation sequencing** using a custom-made amplicon panel, comprising 2778 amplicons covering 53 genes (including single nucleotide polymorphisms and hotspot mutation regions). Samples were multiplexed on an Ion 540 Chip and sequenced on the Ion S5XL Semiconductor Sequencer (Thermo Fisher Scientific). **Somatic mutations** were investigated. The coverage data were explored to identify any **copy number variations**.

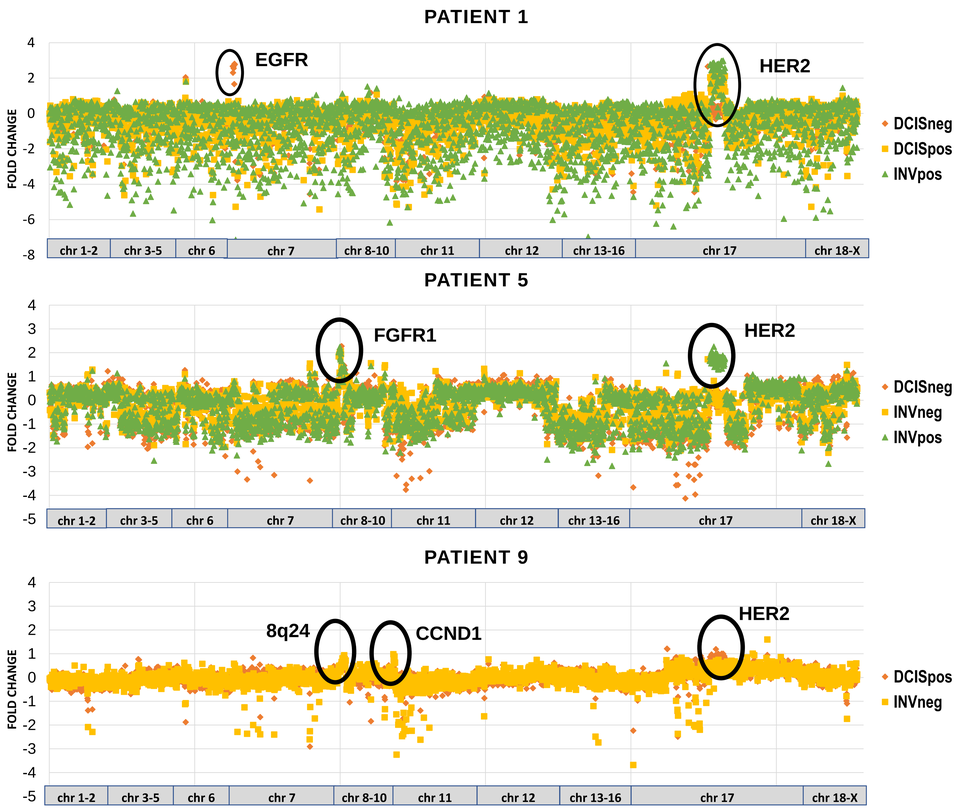
Results

Somatic mutations per patient

We identified 3 splice site alterations, 32 missense variants, 12 deletions, 9 insertions, and 7 nonsense variants in 26 different genes, which are (likely) pathogenic. Overall, these molecular anomalies were heterogeneously distributed among the different tumour components. The HER2-negative tumour components did not yield common alternative drivers.

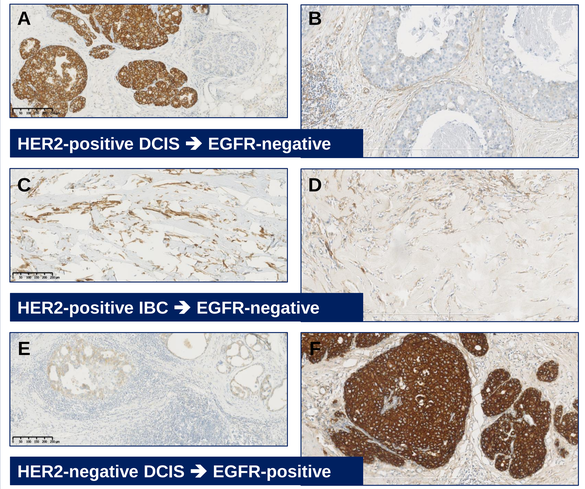


Scatterplots illustrating the presence of copy number variations per patient and per tumour component



One patient had a *CCND1* copy number gain limited to a HER2-negative tumour component. Two patients had an 8q24 gain in at least one component, resulting in increased *MYC* and *PVT1* gene copy numbers. Two patients had an *FGFR1* copy number gain in at least one component. One patient had an *EGFR* copy number gain in a HER2-negative DCIS component, which resulted in EGFR protein overexpression. INV: invasive carcinoma; DCIS: ductal carcinoma in situ.

Immunohistochemistry for HER2 and EGFR in patient 1



Patient 1 had an EGFR copy number gain in the HER2-negative DCIS component (E), which resulted in EGFR protein overexpression (F). The HER2-positive DCIS component (A) and the HER2-positive invasive metaplastic carcinoma (C) did not present with this EGFR copy number gain and were EGFR-negative (B, D). Original magnification 100x.

Conclusion

This series of 10 heterogeneously *HER2*-amplified breast tumours demonstrates that not all breast cancer cells require HER2 as a driver of tumour growth. Several other molecular anomalies are able to act as alternative or collaborative drivers.

This study illustrates that breast carcinogenesis is characterized by a diverse and heterogeneous molecular landscape, of which some genetic anomalies drive cancer progression, and others are mere 'passenger' molecular aberrations.

Want to know more?

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