Early enrichment of ESR1 mutations and the impact on gene expression in primary breast cancer treated with aromatase inhibitors in the pre-surgical setting

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Supplementary figures
338 post-menopausal women with ER+ BC treated with > 1 month NAI

232 patients that signed generic consent for tissue-based research

Diagnostic biopsy or excision surgery specimen not collected at RMH (n=62)

170 paired core-biopsy and excision surgery collected at RMH

Multifocal disease* (n=28)
  Previous BC (<10y; n=5)
  Participation in other neoadjuvant clinical trial (n=21)
  Concomitant anticancer treatmentb (n=7)

109 patients fitting to inclusion and exclusion criteria

Paired blocks not available (n=14)

95 pre-NAI and post-NAI tumour blocks evaluated centrally

<40% invasive cells (n=8)

87 paired tumours with > 40% invasive cells

No generic consent for tissue-based research signed (n=106)

Supplementary Fig. S1. Consort diagram. *Multifocal disease confirmed in histopathology analysis. bConcomitant anticancer treatments included chemotherapy, biologic response modifiers, endocrine therapy (including steroids) and radiotherapy.
Supplementary Fig. S2. Pre- and post-NAI expression of proliferation markers (Ki67 and proliferation metagene), ESR1/ER and ERGs based on clinical response stratification. PAGs: mean of 11 proliferation genes in the PAM50 gene set; ERGs: oestrogen-regulated genes – mean of TFF1, GREB1, PDZK1 and PGR. CR: complete response to therapy (green); PR: partial response (blue); SD: stable disease (yellow); PD: progressive disease (red). Light blue dots mark cases with PR that showed clinical signs of progression disease (>20% increase of the tumour volume in relation to the previous ultrasound). P-values based on T-test are shown.
Supplementary Fig. S3. Correlation between protein and gene expression. A) Ki67, PgR and ER expression measured by NanoString™ technology and IHC. B) Correlation between Ki67 protein expression and PAGs. C) Correlation between PgR protein and ERGs. Individual blue dots mark ESR1 wild-type HER2- tumours, yellow dot ESR1 wild-type HER2+ tumours and red dots ESR1 mutant HER2- tumours. Light colours: pre-NAI values; Dark colours: post-NAI values. P-values and coefficient of correlation (r) based on Pearson correlation test are shown. PAGs: mean of 11 proliferation genes in the PAM50 gene set; ERGs: oestrogen-regulated genes – mean of TFF1, GREB1, PDZK1 and PGR.
Supplementary Fig. S4. E2F activation metagene in NAI-therapy. Overall inhibition of E2F activation metagene with NAI treatment and higher post-NAI expression of this signature in patients with stable disease / progressive disease (SD/PD) in comparison with complete or partial response (CR/PR) in both pre- and post-NAI samples based on clinical response stratification. Arrow graphs represent the individual expression (left) and the mean expression with the 95% confidence interval of the mean difference (right) in pre-NAI and post-NAI samples. P-values based on T-test (box plots) or paired T-test (arrow plots) are shown.
Supplementary Fig. S5. Gene expression based on ESR1 mutational status. A) Pre and post-NAI mean expression of oestrogen-regulated genes (ERGs). Individual values are shown for ESR1 wild-type HER2- tumours (blue bars), ESR1 wild-type HER2+ tumours (yellow bars) and ESR1 mutant HER2- tumours (red bars). Light colours: pre-NAI values; Dark colours: post-NAI values. B) CCND1, RET and FOXM1 expression in ESR1 wild-type (blue dots and arrows) and mutant tumours (red dots and arrows). Less inhibition of these biomarkers was detected in ESR1 mutant tumours. Box plot graphs represent the expression difference (Post-NAI – Pre-NAI) with individual values also shown. Arrow graphs (right) represent the mean expression of each group in pre-NAI and post-NAI samples. P-values based on Mann-Whitney test (box plots) or Wilcoxon (arrow plots) are shown. ERGs: oestrogen-regulated genes – mean of TFF1, GREB1, PDZK1 and PGR. Wt: ESR1 wild-type tumours; Mut: tumours harbouring ESR1 mutation. ESR1 mutation type are highlighted.
Supplementary Fig. S6. Correlation between ERGs and Ki67 expression in ESR1wt tumours. Light blue: low residual Ki67 (% of +ve cells ≤2.7%, n=53). Bright blue: medium level of residual Ki67 (>2.7% & ≤10%, n=15). Dark blue: high residual Ki67 (≥10%, n=13). P-values and coefficient of correlation (r) based on Pearson correlation test are shown.
Supplementary Fig. S7. Change in cyclins expression in ESR1+ tumours classified based on Ki67r. Box plots represent on-treatment change. Arrow graphs (right) represent the mean expression of each group in pre- and post-NAI samples. Light blue: low residual Ki67 (% of +ve cells ≤2.7%, n=53). Bright blue: medium level of residual Ki67 (>2.7% & ≤10%, n=15). Dark blue: high residual Ki67 (≥10%, n=13). P-values and coefficient of correlation (r) based on Pearson correlation test are shown. P-values based on T-test (box plots) or paired T-test (arrow plots) are shown. Ki67r: residual Ki67 (post-neoadjuvant AI therapy).