***ESR1* mutations and overall survival on fulvestrant versus exemestane in advanced hormone receptor positive breast cancer. A combined analysis of SoFEA and EFECT.**

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Supplementary tables and figures

**Supplementary table 1. Baseline characteristics of patients with samples analysed for *ESR1* mutation status in EFECT.**



ER – oestrogen receptor positive; PR – progesterone receptor; ABC – advanced breast cancer; NSAI – non steroidal aromatase inhibitor; AI status – sensitivity to prior aromatase inhibitor (sensitive indicating relapse after at least 2 years adjuvant AI or response or duration of stable disease lasting at least 24 weeks in the metastatic setting). P values from Chi-squared test.

**Supplementary table 2. PFS and OS estimates by treatment group for all EFECT trial patients and patients with *ESR1* mutation status available.**



**Supplementary table 3. Baseline characteristics of patients in SoFEA and EFECT combined analysis.**



ER – oestrogen receptor positive; PR – progesterone receptor; ABC – advanced breast cancer; NSAI – non steroidal aromatase inhibitor; AI status – sensitivity to prior aromatase inhibitor (sensitive indicating relapse after at least 2 years adjuvant AI or response or duration of stable disease lasting at least 24 weeks in the metastatic setting). Treatment allocation – randomisation of EFECT has 1:1 whereas randomisation of SoFEA was 2:1 fulvestrant based therapy versus exemestane. P values from Chi-squared test

**Supplementary Figure 1. Impact of DNA input amount on *ESR1* mutation detection in EFECT**

Total free DNA input amount for digital PCR and detection rates of *ESR1* mutation. DNA extracted from a fixed volume of serum (up to 1ml) was quantified, and DNA equivalent to that extracted from 0.25ml of serum was analysed in each multiplex PCR, capped at a total input of 40ng. Samples with high input DNA amount likely reflect release of genomic DNA from lymphocytes during serum clotting.



**Supplementary Figure 2.** **Consort diagram of patients analysed from EFECT**



**Supplementary Figure 3. Time To Progression in EFECT by detection of *ESR1* mutations in baseline serum**

A. TTP in patients with *ESR1* mutation detected

B. TTP in patients without *ESR1* mutation detected (*ESR1* wild-type).

In patients with *ESR1* mutation detected, time to progression (TTP) was 2.0 months (95%CI,1.7-2.4) on exemestane and 3.5 months (95%CI,1.9-5.0) on fulvestrant (HR=0.67, 95%CI,0.37-1.19, p=0.17). In patients without baseline *ESR1* mutations detected, TTP was 4.5 months (95%CI,3.7-5.6) on exemestane and 3.7 months (95%CI,3.3-5.2) on fulvestrant (HR=1.05, 95%CI,0.75-1.45, p=0.78)



**Supplementary Figure 4. Individual ESR1 mutation detection and polyclonality in SoFEA and in EFECT**

A. *ESR1* mutation distribution in EFECT and SoFEA (p=0.96 chi-square test)

B. *ESR1* polyclonality and monoclonality distribution in EFECT and SoFea (p=0.60 chi-square test)

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**Supplementary Figure 5. Progression free survival by *ESR1* mutation detected in ctDNA**

Progression free survival in combined data set, split by type of *ESR1* mutation detected in ctDNA, D538G, Y537X (Y537S, Y537C, Y537N), and E380Q/S463P. Comparison of exemestane versus fulvestrant. For D538G (N=65 patients) D538G (N=65 patients) HR = 0.53 (95%CI: 0.29, 0.94); p=0.03: Y537X (65 patients) HR = 0.46 (95%CI: 0.27, 0.81); p=0.01: E380Q/S463P (22 patients) HR = 0.56 (95%CI: 0.22, 1.43); p=0.22. Mutation groups were defined prior to association with clinical outcome data, following assessment of *in vitro* data on fulvestrant sensitivity1-4. Patients with polyclonal mutations may be represented in more than one graph. There were insufficient patients to look separately at Y537S.

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**Supplementary Figure 6. Progression free survival in monoclonal and polyclonal ESR1 mutations**

Progression free survival in combined data set, divided by monoclonal (single *ESR1* mutation detected in ctDNA) and polyclonal (two or more *ESR1* mutations detected in ctDNA). Compared with Polyclonal+Exemestane group (n=16): Monoclonal+Fulvestrant (n=44) HR =0.55  (95%CI: 0.31, 0.99); p=0.02: Monoclonal+Exemestane  (n=26) HR =0.67  (95%CI: 0.35, 1.28); p=0.35: Polyclonal+Fulvestrant  (n=26) HR =0.32  (95%CI: 0.16, 0.63); p=0.01

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**References**

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