Background
Selection of resistance mutations may play a major role in the development of endocrine resistance. ESR1 mutations are rare in primary breast cancer but have a high prevalence in patients treated with aromatase inhibitors (AI) for advanced breast cancer. We investigated the evolution of genetic resistance to first line AI therapy using sequential circulating tumour DNA (ctDNA) sampling in patients with advanced breast cancer.

83 patients on first line AI therapy for metastatic breast cancer were enrolled in a prospective study to collect plasma samples for ctDNA analysis every three months on therapy, and at disease progression. All plasma samples were analysed with ESR1 multiplex digital PCR assays, and samples at disease progression were analysed by InVision® (enhanced tagged-amplification sequencing). Mutations were tracked back through samples prior to disease progression, to study the evolution of mutations on therapy. Subclonal ESR1 mutations were defined as mutations with aggregate allele frequency <0.25 of breast cancer driver mutation allele frequency identified in the sample.

Laboratory

"For the 39 patients who progressed on first line AI, 56% (22/39) had ESR1 mutations detectable at progression, which were polyclonal in 40.9% (9/22) patients. In patients with additional driver mutations detected in ctDNA, ESR1 mutations were subclonal in 76.8% (11/14) patients.

Mutations identified in progression plasma DNA by InVision® sequencing, with ESR1 mutation analysis by dPCR. Polyclonal KRAS mutations were identified in two patients, 8005 (p.G12V, p.G12S) and 8032 (p.G12V, p.G12C, p.G12R, and HRAS) in one case. An activating p.R46C FGFR3 mutation was identified in a further patient plasma sample.

Conclusions
ESR1 mutations are found at high prevalence in patients progressing on first line AI for metastatic breast cancer, but are frequently subclonal. KRAS mutations are identified as a putative novel mechanism of resistance to AI, associated with co-detection of ESR1 mutations. AI resistant cancers show genetic diversity that may limit subsequent targeted therapy approaches.