Poster Number:1015

Tracking evolution of aromatase inhibitor resistance with circulating tumor DNA in metastatic breast cancer

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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.

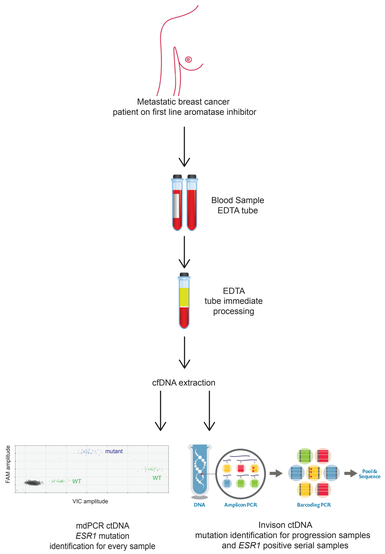
Study design

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.

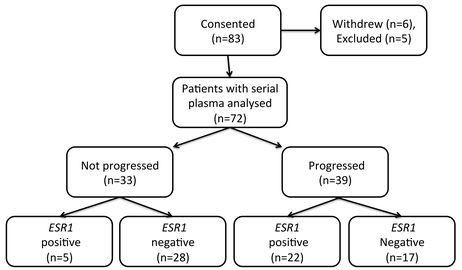
All plasma samples were analysed with *ESR1* multiplex digital PCR assays, and samples at disease progression were analysed by InVision® (enhanced tagged-amplicon sequencing).

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.



Samples analysed in the plasma AI study

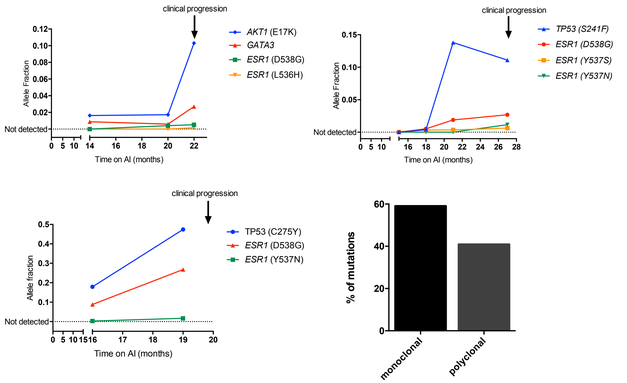


Clinicopathological characteristics of study patients

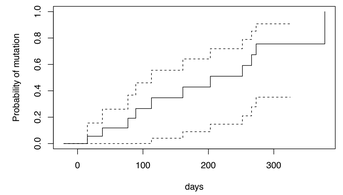
	n	%		n	%
n	72		**Prior adjuvant endocrine therapy**		
Median age	68 (37-94)		Tamoxifen only	40	55.6
Pathology			AI only	7	9.7
IDC	49	68.0	AI and tamoxifen	11	15.3
ILC	16	22.2	none	14	47.2
Mixed IDC/ILC	3	4.2			
NA/other	4	5.6	**Prior chemotherapy**		
Grade			Neo/adjuvant	29	40.3
1	7	9.7	Metastatic +/- adj	6	8.3
2	50	69.4	None	37	51.4
3	8	11.1			
NA	7	2.6	**Metastatic sites**		
Hormone receptor status			Bone	58	80.6
ER positive/PR positive	39	54.2	Visceral	41	56.9
ER positive/PR negative	10	13.9			
ER positive/PR NK	23	31.9			

Mutation tracking in ctDNA: *ESR1* subclones

Of 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 78.6% (11/14) patients.

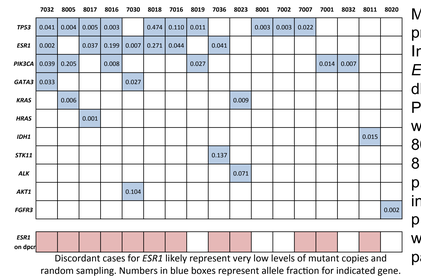


Lead time to development of *ESR1* mutations



In serial tracking prior to progression, *ESR1* mutations were detectable in plasma median 6.7 months (95% CI 3.7-NA) prior to clinical progression.

Genomic profile of AI resistant breast cancer



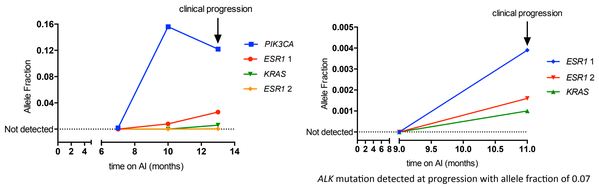
Discordant cases for *ESR1* likely represent very low levels of mutant copies and random sampling. Numbers in blue boxes represent allele fraction for indicated gene.

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 8023 (p.G12V, p.G12C, p.G12R), and *HRAS* (p.G12V) in one case. An activating p.R248C *FGFR3* mutation was identified in a further patient plasma sample.

Mutation tracking in ctDNA: *KRAS* subclones

Sequencing or digital PCR of progression plasma DNA identified *RAS* mutations in 13.3% (4/30) progressing patients (3 polyclonal *KRAS*, 1 monoclonal *HRAS*). All *RAS* mutations were sub-clonal, and were detected in samples with *ESR1* mutations.



ALK mutation detected at progression with allele fraction of 0.07

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.

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