

# Supplementary Information

*Actionable genomic landscape remains stable across serial circulating tumor DNA analyses in advanced breast cancer*

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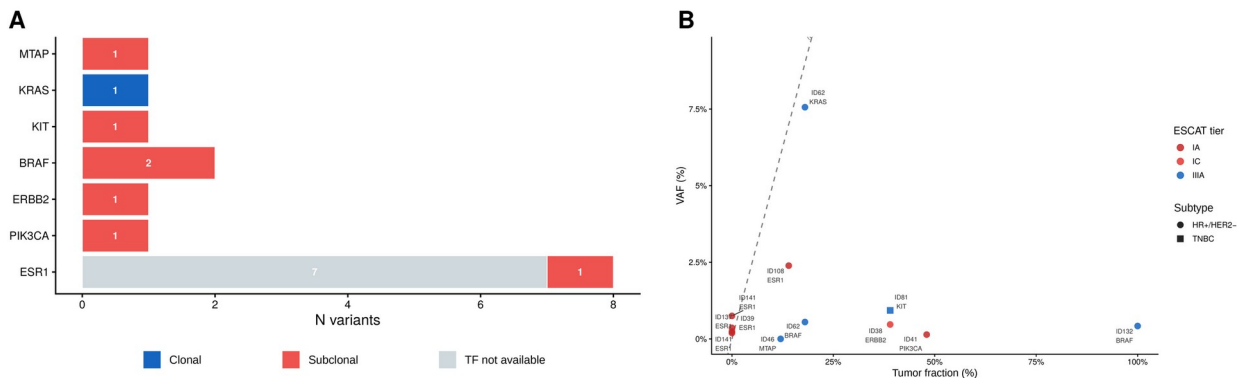
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**Supplementary Table 1 | Technical characteristics of the FoundationOne Liquid CDx assay**

| Assay characteristic                             | FoundationOne Liquid CDx |
|--------------------------------------------------|--------------------------|
| Gene panel                                       | 324 genes                |
| Complete coding exon coverage                    | 309 genes                |
| Selected non-coding coverage                     | 15 genes                 |
| Enhanced-sensitivity bait set                    | 75 genes                 |
| Short variants, enhanced sensitivity, median LoD | 0.40% VAF                |
| Short variants, standard sensitivity, median LoD | 0.82% VAF                |
| Rearrangements, enhanced sensitivity, median LoD | 0.37% VAF                |
| Rearrangements, standard sensitivity, median LoD | 0.90% VAF                |
| Copy-number amplifications, median LoD           | 21.7% TF                 |
| Copy-number losses, median LoD                   | 30.4% TF                 |

Source: FoundationOne Liquid CDx Technical Specifications (SPEC-01746, version 2.0, January 2026).  
LoD, limit of detection; TF, tumor fraction; VAF, variant allele frequency.

Supplementary Figure 1 | Clonality of newly detected ESCAT-classified alterations.



**a**, Number of newly detected ESCAT-classified variants per gene according to inferred clonality: clonal (blue), subclonal (red) or not assessable because tumor fraction (TF) was unavailable (gray). Variants were classified as clonal when the ratio of variant allele frequency (VAF) to  $TF/2$  was  $\geq 0.5$ . **b**, VAF versus TF for newly detected ESCAT-classified variants; each symbol represents a variant, labeled with the patient identifier and gene. Colour indicates the ESCAT tier and shape the subtype (circles, HR+/HER2-; squares, triple-negative breast cancer (TNBC)). The dashed line indicates the expected VAF of a clonal heterozygous mutation in a diploid tumor ( $VAF = TF/2$ ). Variants with unavailable TF are plotted at 0%. ESCAT, ESMO Scale for Clinical Actionability of Molecular Targets; HER2-, human epidermal growth factor receptor 2-negative; HR+, hormone receptor-positive.

**Supplementary Data 1 | Genes covered by the FoundationOne Liquid CDx assay.**

Provided as a separate Excel file. The file lists the 324 genes of the panel in alphabetical order, including the 15 genes with selected non-coding coverage, and the alternative symbols under which some genes are reported by the manufacturer.