Supplementary Information file: Use of ctDNA in early breast cancer: analytical validity and clinical potential

Supplementary Table 1: Criteria for ctDNA positivity of tumor-agnostic assays

Author and ref	ctDNA assay technique	Criteria for ctDNA positivity
Schrag et al[1]	Targeted methylation analysis Galleri™	• Computational algorithm
Cohen et al[2], Lennon et al[3]	Amplicon-based NGS CancerSEEK	• The mutation must be in the COSMIC database or inactivate a tumor suppressor gene • Statistical confirmation and control on WBC DNA to exclude CHIPs when a mutation is detected
Stecklein et al[4]	Amplicon-based NGS of 275 genes related to cancer	• Tumoral VAF ≥ 3% • Patients with mutations only in *DNMT3A*, *TET2*, *ASXL1*, or *JAK2* were classified as ctDNA-negative as most likely arising from CHIPs
Radovitch et al[5,6]	Hybridization-based targeted NGS of cancer-related genes FoundationACT™ or FoundationOneLiquid™	• Statistical model used for calling a ctDNA sample positive
Li S. et al[7-9]	Hybridization-based NGS of 1021 genes	• CHIPs mutation filtering, excluding mutations in *DNMT3A, IDH1,* and *IDH2* and specific alterations within *ATM, GNAS*, and *JAK2* • No clear criteria for ctDNA positivity were given
Lin P.H. et al[10]	Tumor-agnostic Amplicon-based NGS	• Presence of a pathogenic or likely pathogenic mutation according to the American College of Medical Genomics and Genetics (ACMG) guidelines

		• Tumor-sequencing in some case to confirm the tumoral origin of mutation
Janni et al[11,12]	Hybridization-based NGS and methylation analysis GuardantReveal™	• Bioinformatics pipeline software, trained to detect the presence of ctDNA based on multiple analytic features, including genomic variation (single-nucleotide variants and insertion-deletion alterations) and epigenomic signals, and to exclude common sources of interference such as CHIPs
Elliott et al[13,14]	Tumor-agnostic Methylation analysis from GuardantINFINITY™	• Bioinformatics pipeline software trained to detect cancer based on epigenomic signals

Abbreviations: ctDNA: circulating tumor DNA, CHIPs: Clonal hematopoiesis of indeterminate potential, NGS: next-generation sequencing, SNV: single nucleotide variation, VAF: Variant allele frequency WBC: white blood cell

Supplementary Table 2: Criteria for ctDNA positivity of tumor-informed assays

Author and ref	ctDNA assay technique	Number of somatic mutations followed	Criteria for ctDNA positivity
Cavallone et al[15], Roseshter et al[16], Basik et al[17]	ddPCR	5	Two standard deviations above the control (assay conducted on the plasma of three healthy donors)
Garcia-Murillas et al[18,19], Turner et al (c-TRAK TN)[20]	ddPCR	1 or 2	≥ 2 positive droplets. • To confirm a positive result, it was repeated on a 2nd sample from the same timepoint in the c-TRAK TN trial
Ciriaco et al[21]	Amplicon-based NGS via Sysmex SafeSEQ	1 to 6	≥ 3 copies/mL and 3 times the value of the background established for each variant (from commercial healthy genomic DNA)

Parsons et al (TBCRC 030 trial)[22,23]	hybridization-based NGS MAESTRO	319 to 1000 (Median 1000)	Predefined 90% power. If less than 10 mutations were identified, they were reviewed manually
McDonald et al[24]	amplicon-based NGS TARDIS	6 to 115 (average 30)	• Minimum 2 read families (RFs) • For each mutation: at least 0.5 mutant copies/reaction (mutant RFs / total RFs) • If only one variant is present: at least two different RF lengths Each sample-level positive ctDNA result was confirmed using the Bonferroni corrected p-value <0.05
Rothé et al[25]	ddPCR	1	Statistically higher than the control (mean of 8 assays conducted on mutation-negative cell lines or healthy donors)
Riva et al[26]	ddPCR	1	≥ 2 positive droplets • Cut-off established testing their assay on a minimum of 5 control DNA for each variant. Specificity 99.4%
Zhou et al[27]	ddPCR	average 2.6 mutations	Tumoral VAF ≥ 0.1% • Established testing their assay on commercial mutated DNA for some variants
Ortolan et al[28], La Rocca et al[29]	ddPCR	1	≥ 3 positive droplets in all replicates
Chen Y.-H. et al[30]	Amplicon-based targeted NGS.	Not applicable	Only mutation(s) present(s) in the primary tumor were considered ctDNA-positive
Takahashi et al[31]	OS-MSP of *RASSF1A*	1 (methylated gene)	≥ 3.3 copies/mL • Established testing their assay on commercial fully methylated DNA
Magbanua et al[32], Cailleux et al[33], Shaw et al[34],	Signatera™	16	• ≥ 2 variants present with a confidence score above a predefined algorithm threshold (0.97)

			• Exclude mutations from CHIPs (from sequencing the buffy coat and bioinformatic pipeline)
Lipsyc-Sharf et al[35]	RaDaR™	Up to 48	• A statistical model is used to assess the statistical significance of the observed mutant counts for each variant to consider a sample positive or negative • Exclude mutations from CHIPs (from sequencing the buffy coat and bioinformatic pipeline)

Abbreviations: 2nd: second, CHIPs: Clonal hematopoiesis of indeterminate potential, ctDNA: circulating tumor DNA, ddPCR: digital drop polymerase chain reaction, mL: milliliter, NGS: next-generation sequencing, RF: read family, VAF: Variant allele frequency

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