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The diagnostic accuracy of *PIK3CA* mutations by circulating tumor DNA in breast cancer: an individual patient data meta-analysis

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Abstract

Background: The circulating tumor DNA (ctDNA) diagnostic accuracy for detecting phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations in breast cancer (BC) is under discussion. We aimed to compare plasma and tissue *PIK3CA* alterations, encompassing factors that could affect the results.

Methods: Two reviewers selected studies from different databases until December 2020. We considered BC patients with matched tumor tissue and plasma ctDNA. We performed meta-regression and subgroup analyses to explore sources of heterogeneity concerning tumor burden, diagnostic technique, sample size, sampling time, biological subtype, and hotspot mutation. Pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and the related area under the curve (AUC) were elaborated for the overall population and each subgroup.

Results: The pooled analysis was carried out on 25 cohorts for a total of 1966 patients. The overall ctDNA sensitivity and specificity were 0.73 (95% CI: 0.70–0.77) and 0.87 (95% CI: 0.85–0.89). The AUC was 0.93. Pooled concordance, negative predictive value and positive predictive value values were 0.87 (95% CI: 0.82–0.92), 0.86 (95% CI: 0.81–0.90), and 0.89 (95% CI: 0.81–0.95) with pooled PLR, NLR, and DOR of 7.94 (95% CI: 4.90–12.86), 0.33 (95% CI: 0.25–0.45), and 33.41 (95% CI: 17.23–64.79), respectively. The pooled results consistently favored next-generation sequencing (NGS)- over polymerase chain reaction-based methodologies. The best ctDNA performance in terms of sensitivity, specificity, and AUC (0.85, 0.99, and 0.94, respectively) was observed in the low-time sampling subgroup (⩽18 days between tissue and plasma collection). Meta-regression and subgroup analyses highlighted sampling time as a possible major cause of heterogeneity.

Conclusions: These findings reliably estimate the high ctDNA accuracy for the detection of *PIK3CA* mutations. A ctDNA-first approach for the assessment of *PIK3CA* mutational status by NGS may accurately replace tissue tumor sampling, representing the preferable strategy at diagnosis of metastatic BC in patients who present with visceral involvement and at least two metastatic lesions, primarily given low clinical compliance or inaccessible metastatic sites.

Keywords: breast cancer, ctDNA, diagnostic accuracy, meta-analysis, *PIK3CA*

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Background

The onset of somatic activating mutations of the phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) has been associated with acquired resistance to endocrine therapy (ET) in ~40% of advanced hormone-positive (H+) HER2-negative (HER2−) breast cancer (BC) cases.[1–4] Encouraging results regarding

the use of the PIK3CA inhibitor alpelisib with ET for relapsed or progressed BC patients have been reported, confirming the predictive role of *PIK3CA* mutations in this setting.[5–7] Although tissue biopsy is considered the gold standard for prognostic and predictive information, a high concordance rate between tissue and liquid biopsy has been reported in different histotypes.[8–12] Several studies demonstrated that the detection of *PIK3CA* mutations using circulating tumor DNA (ctDNA) might represent a reliable option to suggest a better tailored therapeutic strategy.[2] In this regard, the Food and Drug Administration (FDA) has approved the liquid biopsy-based FoundationOne Liquid CDx test (Foundation Medicine, Inc., Cambridge, Massachusetts) as a companion diagnostic for alpelisib.

Nonetheless, the ctDNA diagnostic accuracy in detecting *PIK3CA* mutations is under discussion while not broadly endorsed by all the regulatory agencies.[13] Therefore, we performed a systematic review of the literature and an individual patient data meta-analysis that comprised studies evaluating the ctDNA diagnostic accuracy for detecting *PIK3CA* mutations compared to reference tissue biopsy. We aimed to provide a comparative analysis between plasma and tissue, discussing the pre-analytical and analytical factors that could affect the results.

Methods

Search strategy and study selection

We performed a systematic review of the literature reports on paired tumor tissue and blood samples to estimate ctDNA diagnostic accuracy in evaluating the *PIK3CA* mutational status in BC patients. We reviewed studies published up to 31 December 2020 through Medline (PubMed), EMBASE databases, and Cochrane Library using the following terms: 'breast cancer', 'BC', 'breast', 'phosphoinositide 3-kinase', '*PIK3CA*', 'tissue', 'liquid', 'blood' (Supplemental Figure 1). Furthermore, we examined abstracts presented at the American Society of Clinical Oncology, the European Society for Medical Oncology, and the San Antonio Breast Cancer Symposium meetings. We searched for unpublished data reported on https://www.clinicaltrials.gov. Restriction for human studies and the English language was applied. We selected records meeting the following inclusion criteria: (1) patients with a histologically confirmed diagnosis of either early (stages I/II/III) or advanced (stage IV) BC; (2) studies detecting *PIK3CA* pathogenic variants in tissue and plasma samples; and (3) studies testing *PIK3CA* mutations by plasma ctDNA analysis. Studies not matching the inclusion criteria and ongoing clinical trials were excluded from the analysis. Only plasma ctDNA data from mixed plasma/serum cohorts were considered. When a study encompassed various follow-ups, we picked up the most recent one. The search protocol was registered in the PROSPERO 2021 database with the code: CRD42020222096.

Data extraction and assessment of the quality of the included studies

Two authors (L.C. and V.G.) independently assessed data extraction and examination. Disagreements were solved by consulting a third author (A.G.). Information retrieved from the included studies comprised: first author name, year of publication, study design, number of patients, biological subtype, study treatment, tumor burden (stage, number of metastatic lesions, and visceral and non-visceral disease), site (primitive or metastasis), diagnostic technique [polymerase chain reaction (PCR), digital droplet PCR (ddPCR), beads, emulsions, amplification, and magnetics (BEAMing), and next-generation sequencing (NGS)] with the limit of detection and *PI3K* reference range, ctDNA mutant allele fraction (MAF), sampling time, number of true-positives (TPs), true-negatives (TNs), false-positives (FPs), and false-negatives (FNs) (Supplemental Tables 1–7). The meta-analysis was designed according to the PRISMA guidelines (Supplemental Figure 1).[14–17] Two authors (L.C. and V.G.) separately assessed the qualitative and quantitative analysis of the studies according to the QUAlity of Diagnostic Accuracy Studies 2 (QUADAS-2) tool,[18] considering four domains: patient selection, index test, reference standard, and flow and timing. The risk of selective outcome reporting bias was investigated, and divergences were overcome by consensus.

Statistical analysis

We extracted data considering the evaluation of *PIK3CA* mutational status on tissue as the gold standard and on ctDNA as the experimental procedure (Supplemental Table 2). The following rates were calculated: sensitivity, specificity, concordance, negative predictive value (NPV), positive predictive value (PPV), positive likelihood

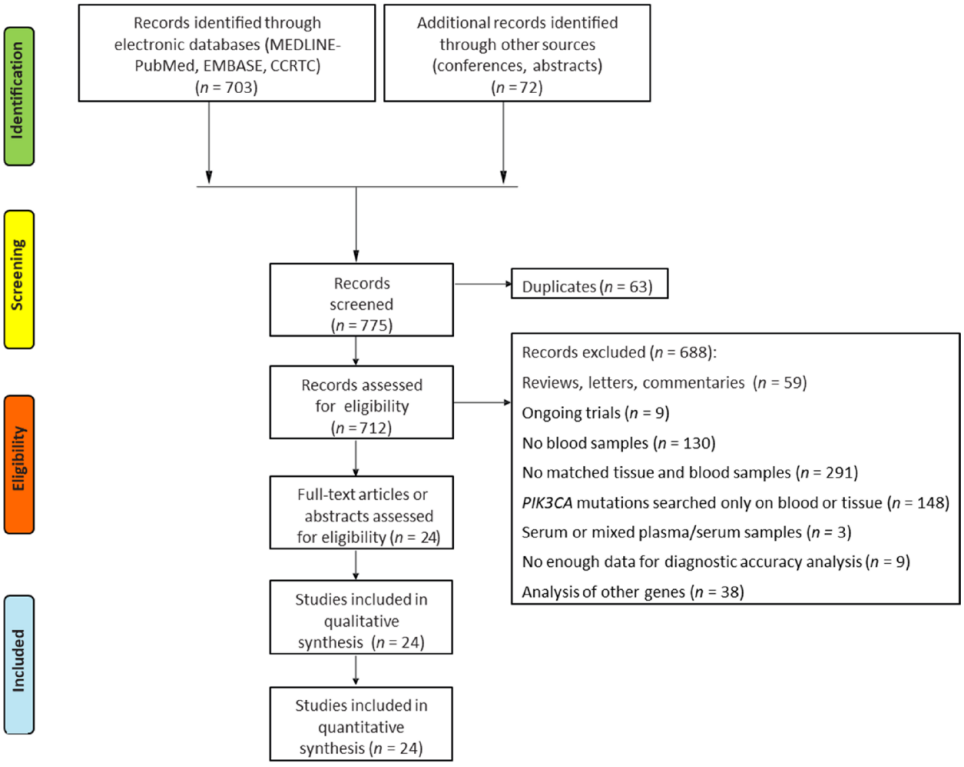

Figure 1. PRISMA flow diagram of the studies included in the quantitative synthesis.

ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and the respective 95% CI (Supplemental Table 6). The random effect DerSimonian Laird model, evaluating the variance between studies, was used to pool PLR, NLR, and DOR.[19] A summary receiver operating characteristics (sROC) curve and the area under the curve (AUC) calculation were elaborated. Meta-regression and differing subgroup analyses were performed to explore heterogeneity concerning disease stage, diagnostic technique, sample size, sampling time, biological subtype [H+/Her2− *versus* HER2-positive (HER2+)], and hotspot mutations (E542/545X *versus* H1047X). We considered the median days between tissue and plasma collection to divide patients into low- and high-time subgroups. Fagan's nomogram was produced to identify the association between pre-test probability, likelihood ratio, and post-test probability.[20] Spearman's rank correlation coefficient between sensitivity and 1-specificity logit evaluated the bias connected to the threshold effect. A p-value <0.05 was considered a significant bias produced by the threshold effect. A p-value of Cochran's Q test <0.05 and an index of inconsistency (I^2) $>50\%$ were considered associated with significant heterogeneity within and between studies, respectively. We used STATA software (StataCorp. *Stata statistical software: release 15*. College Station, TX: StataCorp LLC, 2017)[21] to investigate publication bias producing Deek's plot for asymmetry. All analyses were performed using the MetaDisc statistical software (version 1.4).[22]

Results

The systematic review of the literature provided 775 records. After screening and eligibility assessment, 24 studies met the inclusion criteria. Namely, one trial contained both prospective and retrospective cohorts: this was analyzed as two separate datasets.[23] The pooled analysis was finally carried out on 25 cohorts for a total of 1966 patients (Figure 1). The main features of selected studies are summarized in Table 1 and Supplemental Table 1.

Overall diagnostic accuracy analysis

Across the included studies, sensitivity ranged from 25 to 100%, specificity from 69 to 100%,

Table 1. Comparison of tissue *versus* ctDNA for detection of *PIK3CA* mutations according to technique and performance.

Study	Sample	Methodology	Reference range (*PIK3CA*)	Mutation	Cross-tab analysis				Diagnostic accuracy	%
Chung *et al.*[24]	Tissue	Hybrid capture-based NGS (Hi-Seq, Illumina) (Foundation Medicine)	NA	H1047L (1); H1047R (1); E545K (1)		Tissue +	Tissue –	Total	Sensitivity	100
					ctDNA+	3	0	3	PPV	100
	Plasma	Hybrid capture-based NGS (Hi-Seq, Illumina) (FoundationACT ctDNA assay)	E542K; E545K; H1047R; H1047L	E545K (1); H1047L (1); H1047R (1); E726K (1)	ctDNA–	0	11	11	Specificity	100
					Total	3	11	14	NPV	100
									Concordance	100
Baselga *et al.*[25]	Tissue	Sanger Sequencing	R88Q R93Q/W K111E/N G118D E365K C420R E542K E545G/K Q546K H1047R/K/Y	NA		Tissue +	Tissue –	Total	Sensitivity	71.2
					CtDNA+	99	64	163	PPV	60.7
	Plasma	BEAMing		NA	ctDNA–	40	243	283	Specificity	79.2
					Total	139	307	446	NPV	85.9
									Concordance	76.7
Chae *et al.*[26]	Tissue	NGS (Guardant360 and FoundationOne testing)	NA (indel/point mutation)	NA		Tissue +	Tissue –	Total	Sensitivity	25
					ctDNA+	3	2	5	PPV	60
	Plasma			NA	ctDNA–	9	31	40	Specificity	93.9
					Total	12	33	45	NPV	77.5
									Concordance	75.6
Board *et al.*[27]	Tissue	RT-PCR (ARMS primers/ Scorpion probes)	E542K; E545K; H1047R; H1047L	E542K (3); E545K (9); H1047R (10); H1047L (2)		Tissue +	Tissue –	Total	Sensitivity	33.3
					ctDNA+	8	1	9	PPV	88.9

(Continued)

Table 1. (Continued)

Study	Sample	Methodology	Reference range (*PIK3CA*)	Mutation	Cross-tab analysis				Diagnostic accuracy	%
	Plasma			E542K (1); E545K (6); H1047R (4); H1047L (2)	ctDNA–	16	46	62	Specificity	97.9
					Total	24	47	71	NPV	74.2
									Concordance	76.1
Dawson *et al.*[28]	Tissue	NGS (HiSeq, llumina) (paired-end sequencing)	Selected regions (TAm-Seq)	E545K (6); H1047L (1); H1047R (4); E545K+ H1047R(1)		Tissue +	Tissue –	Total	Sensitivity	100
					ctDNA+	12	0	12	PPV	100
	Plasma	dPCR; NGS (HiSeq, llumina) (paired-end sequencing)	NA; selected regions (TAm-Seq)	Exon 10 (6); Exon 21 (5); Exon 10 + Exon 21 (1)	ctDNA–	0	18	18	Specificity	100
					Total	12	18	30	NPV	100
									Concordance	100
Higgins *et al.*[23] (p)	Tissue	PCR [Pyromark Q24 (Qiagen)], BEAMing (Inostics GmbH)	E542K; E545K; H1047R	E542K (2); E545K (2); H1047R (10)		Tissue +	Tissue –	Total	Sensitivity	57.1
					ctDNA+	8	8	16	PPV	50
	Plasma	BEAMing (Inostics GmbH)	E542K; E545K; H1047R	E542K (3); E545K (2); H1047R (10); E545K + H1047R (2)	ctDNA–	6	29	35	Specificity	78.4
					Total	14	37	51	NPV	82.9
									Concordance	72.5
Higgins *et al.*[23] (r)	Tissue	BEAMing	E545K; H1047R; H1047L	E545K (3); H1047R (10); H1047L (1)		Tissue +	Tissue –	Total	Sensitivity	100
					ctDNA+	14	0	14	PPV	100
	Plasma				ctDNA–	0	35	35	Specificity	100
					Total	14	35	49	NPV	100
									Concordance	100
Rothe *et al.*[29]	Tissue	NGS (Ion Torrent; Illumina)	NA (Ion AmpliSeq™ Cancer Hotspot Panel v2)	H1047R (1) H1047L (3) E453K (2)		Tissue +	Tissue –	Total	Sensitivity	75

(Continued)

Table 1. (Continued)

Study	Sample	Methodology	Reference range (*PIK3CA*)	Mutation	Cross-tab analysis				Diagnostic accuracy	%
					ctDNA+	3	1	4	PPV	75
	Plasma			H1047R (1) E453K (3) H1047L (1)	ctDNA–	1	12	13	Specificity	92.3
					Total	4	13	17	NPV	92,3
									Concordance	88.2
García-Saenz *et al.*[30]	Tissue	RT-PCR (COBAS® PIK3CA Mutation Test; TaqMan assays on the QuantStudio® 3D Digital PCR System); ABI 3130 genetic analyzer	R88Q; N345K; C420R; E542K; E545X (E545A, E545D, E545G, and E545K); Q546X (Q546E, Q546K, Q546L, and Q546R); M1043I; H1047X (H1047L, H1047R, and H1047Y); G1049R	E542K (4); E545K (5); H1047R (11)		Tissue +	Tissue –	Total	Sensitivity	100
					ctDNA+	11	0	11	PPV	100
	Plasma	dPCR (QuantStudio® 3D Digital PCR System)	E542K; E545K; H1047R	E542K (2); E545K (4); H1047R (5)	ctDNA–	9	29	38	Specificity	100
					Total	20	29	49	NPV	76.3
									Concordance	81.6
Shatsky *et al.*[31]	Tissue	NGS (The FoundationOne genomic assay)	NA	H1047R (3); E542K (1); E545K (2); Q75E (1)		Tissue +	Tissue –	Total	Sensitivity	77.8
					ctDNA+	7	1	8	PPV	87.5
	Plasma	NGS (The Guardant 360 assay)	NA	H1047R (5); Amplification (2); R108H (1); E542K(1); E545K (4); H1047Q + E545K (1); E81K (1); E453K (1)	ctDNA–	2	28	30	Specificity	96.6
					Total	9	29	38	NPV	93.3
									Concordance	92.1
Spoerke *et al.*[32]	Tissue	RT-PCR BEAMing (OncoBEAM BC1 BEAMing Digital PCR panel)	C420R, E542K, E545A/G/K, and H1047L/R/Y	H1047R (16); H1047R (8); H1047R + E545K (1); H1047L + E542K + E545K (1)		Tissue +	Tissue –	Total	Sensitivity	78.1

(Continued)

Table 1. (Continued)

Study	Sample	Methodology	Reference range (*PIK3CA*)	Mutation	Cross-tab analysis				Diagnostic accuracy	%
					ctDNA+	50	7	57	PPV	87.7
	Plasma		C420R, E542K, E545K/G, Q546K, M1043I, and H1047R/L/Y		ctDNA–	14	71	85	Specificity	91
					Total	64	78	142	NPV	83.5
									Concordance	80.5
Tzanikou *et al.*[33]	Tissue	ddPCR	E545K; H1047R	E545K (2); H1047R (1); E545K + H1047R (7)		Tissue +	Tissue –	Total	Sensitivity	38.5
					ctDNA+	5	2	7	PPV	71.4
	Plasma			E545K (1); E545K + H1047R (5)	ctDNA–	8	1	9	Specificity	33.3
					Total	13	3	16	NPV	11.1
									Concordance	37.7
Bianchini *et al.*[34]	Tissue	NGS (AmpliSeq HD, Oncomine Pan-Cancer)	NA	NA		Tissue +	Tissue –	Total	Sensitivity	46.6
					ctDNA+	27	2	29	PPV	93
	Plasma				ctDNA–	31	84	115	Specificity	97.7
					Total	58	86	144	NPV	73
									Concordance	77.1
Oliveira *et al.*[35]	Tissue	Amplicon NGS based (MiSeq Illumina)	NA (59 cancer panel genes)	NA		Tissue +	Tissue –	Total	Sensitivity	76.2
					ctDNA+	16	0	16	PPV	100
	Plasma	Amplicon NGS based (MiSeq Illumina)	NA	NA	ctDNA–	5	1	6	Specificity	100
					Total	21	1	22	NPV	16.7
									Concordance	77.3
Di Leo *et al.*[36]	Tissue	COBAS	NA (*PIK3CA* assay covering exons 7, 9, and 20)	NA		Tissue +	Tissue –	Total	Sensitivity	80.5

(Continued)

Table 1. (Continued)

Study	Sample	Methodology	Reference range (*PIK3CA*)	Mutation	Cross-tab analysis				Diagnostic accuracy	%
					ctDNA+	70	21	91	PPV	76.9
	Plasma	BEAMing	E542K E545G/KQ546K M1043I H1047L/R/Y	NA	ctDNA−	17	142	159	Specificity	87.1
					Total	87	163	250	NPV	89.3
									Concordance	84,8
Blackwell *et al.*[37]	Tissue	Hybridization-captured NGS based	Foundation Medicine, Inc.	N345 K (2), C420R (2) E542 K (2), E545 K (1), Q546 K (1) H1047R (10), H1047L (2)		Tissue +	Tissue −	Total	Sensitivity	94.4
					ctDNA+	17	1	18	PPV	94.4
	Plasma	BEAMing	E542K, E545K, E545G, Q546K; M1043I; H1047L; c.3139C>T_p. H1047R; c.3140A> G_p.H1047R	N345 K (1), C420R (1) E542 K (2), E545 K (1), Q546 K (1) H1047R (10), H1047L (2)	ctDNA−	1	12	13	Specificity	92.3
					Total	18	13	31	NPV	92.3
									Concordance	93,5
Moynahan *et al.*[38]	Tissue	NGS (HiSeq, Illumina)	NA	NA		Tissue +	Tissue −	Total	Sensitivity	73.3
					ctDNA+	63	50	113	PPV	55.8
	Plasma	ddPCR	E542K; E545K; H1047R	E542K (39); E545K (61); H1047R (138): multiple[a]: (4) [a]Three E545K/E542	ctDNA−	23	111	134	Specificity	68.9
					Total	86	161	247	NPV	82.9
									Concordance	70.4
Moreno *et al.*[39]	Tissue	NGS (Ion Torrent; Illumina)	NA (a customized panel of 54 genes)	H1047R (7) A511T (1) V344G (1) Va46G (1) A668C (1) G106V (1) T462A (1) G451_D54del (1) C420R (1)		Tissue +	Tissue −	Total	Sensitivity	72.7

(Continued)

Table 1. (Continued)

Study	Sample	Methodology	Reference range (*PIK3CA*)	Mutation	Cross-tab analysis				Diagnostic accuracy	%
					CtDNA+	8	0	8	PPV	100
	Plasma	NGS (Ion Torrent; Illumina)	NA (a customized panel of 54 genes)	H1047R (7) A511T (1) V344G (1) V346G (1) A668C (1) G106V (1) T462A (1) C420R (1)	ctDNA–	3	27	30	Specificity	100
					Total	11	27	38	NPV	90
									Concordance	92.1
Takano *et al.*[40]	Tissue	ddPCR	E542K, E545K, H1047R	E542K (2); E545K (1); H1047R (10)		Tissue +	Tissue –	Total	Sensitivity	60
					ctDNA+	6	0		PPV	100
	Plasma			H1047R (8)	ctDNA–	4	16	20	PPV	100
					Total	10	16	26	Specificity	100
									NPV	80
									Concordance	84.7
Slembrouck *et al.*[41]	Tissue	NGS	NA	E542K (1); E545K (6); H1047R (1); No hotspot mutation (12)		Tissue +	Tissue –	Total	Sensitivity	100
					ctDNA+	8	0	8	PPV	100
	Plasma	ddPCR	E542K, E545K, H1047R, H1047L	E542K (3); E545K (6); H1047R (1); No hotspot mutation (12)	ctDNA–	0	12	12	Specificity	100
					Total	8	12	20	NPV	100
									Concordance	100
Rudolph *et al.*[42]	Tissue	NGS	Mutations, deletions, amplifications (FoundationOne® T5a panel)	NA		Tissue +	Tissue –	Total	Sensitivity	100
					ctDNA+	13	0	13	PPV	100

(Continued)

Table 1. (Continued)

Study	Sample	Methodology	Reference range (*PIK3CA*)	Mutation	Cross-tab analysis				Diagnostic accuracy	%
	Plasma	BEAMing	E542K, E545K, H1047R, H1047L	NA	ctDNA–	0	37	37	Specificity	100
					Total	13	37	50	NPV	100
									Concordance	100
Perkins *et al.*[43]	Tissue	PCR; MALDI-TOF (OncoCarta panel)	NA	H1047R (4)		Tissue +	Tissue –	Total	Sensitivity	75
					ctDNA +	3	0	3	PPV	100
	Plasma	MALDI-TOF (OncoCarta panel)	NA	H1047R (3)	ctDNA –	1	15	16	Specificity	100
					Total	4	15	19	NPV	93.8
									Concordance	100
Ma *et al.*[44]	Tissue	NGS	NA	NA		Tissue +	Tissue –	Total	Sensitivity	50
					ctDNA+	3	0	3	PPV	100
	Plasma				ctDNA–	3	6	9	Specificity	100
					Total	6	6	12	NPV	66.7
									Concordance	75
Kim *et al.*[45]	Tissue	RT-PCR	C420R; E542K; E545A/G/K; H1047L/R/Y			Tissue +	Tissue -	Total	Sensitivity	100
					ctDNA+	54	0	54	PPV	100
	Plasma	RT-PCR; NGS (Foundat ion One)	R88Q; N345K; C420R; E542X; E545X; Q546X; M1043I; H1047X; G1049R; AKT1	E542K (2); G1049R (1); H1047L (2); H1047R (10); M1043I (1); N345K (1); Q546K (1)	ctDNA–	0	18	18	Specificity	100
					Total	54	18	72	NPV	100
									Concordance	100
Beaver *et al.*[46]	Tissue	Sanger Sequencing ddPCR (Custom TaqMan probes)	E545K; H1047R	E545K (3); H1047R (7) E545K (4); H1047R (10)					Sensitivity	92,9

(Continued)

Table 1. (Continued)

Study	Sample	Methodology	Reference range (*PIK3CA*)	Mutation	Cross-tab analysis			Diagnostic accuracy	%
					ctDNA+	13	0	13	PPV	100
	Plasma				ctDNA–	1	15	16	Specificity	100
					Total	14	15	29	NPV	93,8
									Concordance	96,6

ARMS, amplification-refractory mutation system; BEAMing, beads, emulsions, amplification, and magnetics; ctDNA, circulating tumor DNA; ddPCR, digital droplet polymerase chain reaction; dPCR, digital polymerase chain reaction; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; NA, not available; NGS, next-generation sequencing; NPV, negative predictive value; PCR, polymerase chain reaction; PIK3CA, phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction.

and concordance from 37 to 100% with lower rates being associated with early BC.[33] The pooled ctDNA sensitivity and specificity of ctDNA were 0.73 (95% CI: 0.70–0.77) and 0.87 (95% CI: 0.85–0.89) (Figure 2(a) and (b)). The AUC resulting from the sROC curve was 0.93 (Figure 2(c)). According to Youden's index, the best pooled cut-off able to minimize the FP was 0.6.[47] We obtained pooled concordance, NPV, and PPV equal to 0.87 (95% CI: 0.82–0.92), 0.86 (95% CI: 0.81–0.90), and 0.89 (95% CI: 0.81–0.95), respectively. Pooled PLR, NLR, and DOR were 7.94 (95% CI: 4.90–12.86), 0.33 (95% CI: 0.25–0.45), and 33.41 (95% CI: 17.23–64.79) (Table 2). Assuming a pre-test probability of 37%, Fagan's plot showed that detecting a ctDNA *PIK3CA* mutation would raise the post-test chance to diagnose a tissue *PIK3CA* mutation to 77%, whereas the missed identification would decrease the post-test probability to 15% (Supplemental Figure 2).

Quality analysis and publication bias

Based on the QUADAS-2 results, the records were overall affected by a low risk of bias, increasing the strength of scientific evidence of the study. Only one study (Perkins *et al.*[43]) presented a high risk of bias in the patient selection task since the authors did not include patients tested with negative tissue results (Supplemental Figure 3). The presence of publication bias was explored through Deek's funnel plot, showing a potential risk ($p = 0.04$) (Supplemental Figure 2).

Threshold effect and heterogeneity

Spearman's rank correlation coefficient was −0.276 (p-value = 0.181), thus not significantly associated with bias. Considering the positive publication bias, we performed meta-regression and subgroup analysis to explore sources of heterogeneity not linked to the threshold effect. The meta-regression demonstrated that sampling time was significantly associated with heterogeneity (Supplemental Table 1b).

Subgroup analysis

Furthermore, as a means of investigating heterogeneous results while answering specific clinical questions, we split participant data into subgroups according to tumor burden, sample size,

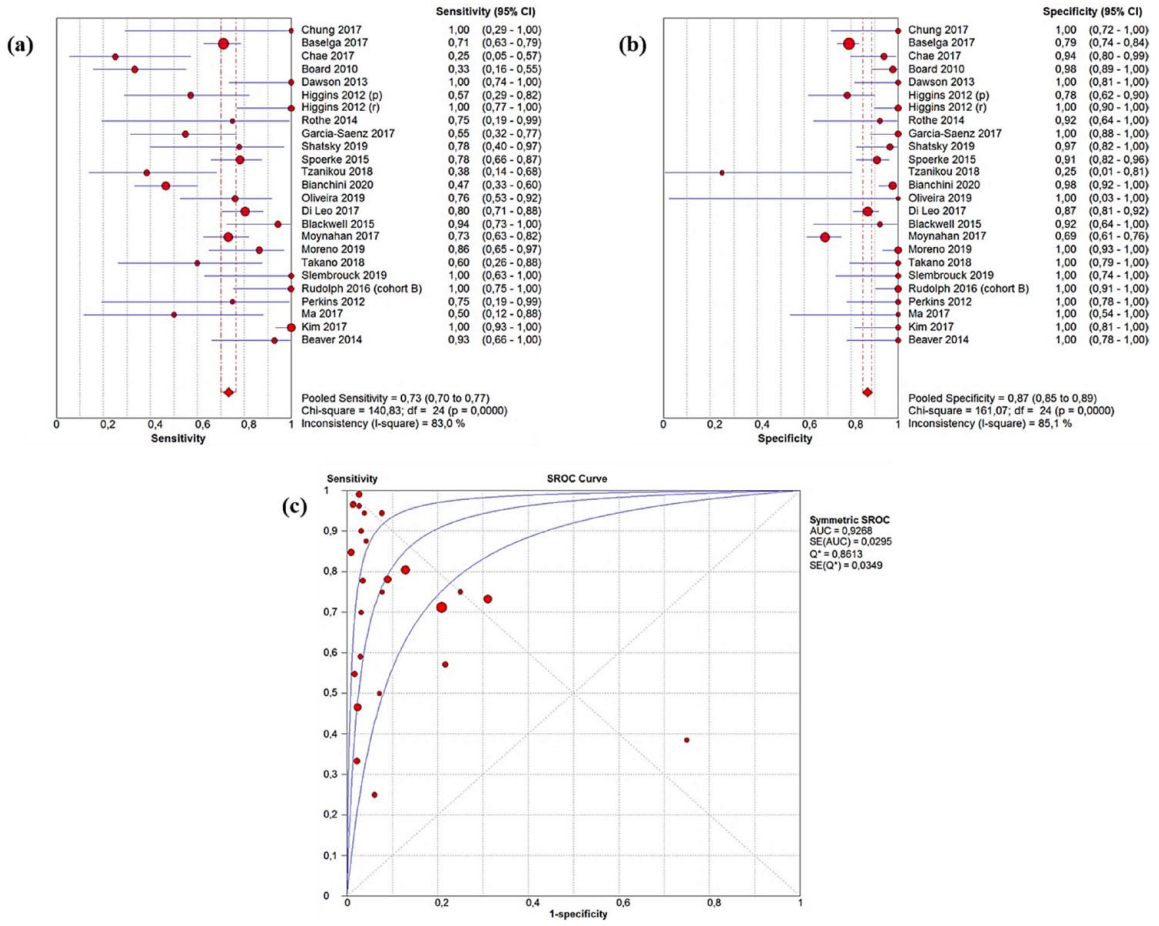

Figure 2. Pooled ctDNA sensitivity (a), specificity (b), and sROC curve related to the overall population (c).
ctDNA, circulating tumor DNA; sROC, summary receiver operating characteristics.

diagnostic technique, sampling time, biological subtype, and hotspot mutation (Table 2).

Tumor burden. Extracting data from cohorts singly evaluating different disease stages, 4 and 23 cohorts were finally assigned to early and advanced subgroups for a total of 55 and 1836 patients, respectively (Supplemental Table 3).[23–46] Regarding the advanced setting, we observed an AUC of 0.92, which showed an excellent discrimination ability between mutated and wild-type patients (Supplemental Figure 4 and Table 2). Furthermore, even if not evaluated in terms of diagnostic accuracy due to missing data, we investigated both the disease distribution and the number of metastatic lesions from nine and eight cohorts, respectively.[23,25,28–30,32,34–36,38,43,44] Most of the examined population had a visceral involvement and at least two metastatic lesions (Supplemental Table 5). Likewise, we found comparable pooled diagnostic values for the early subgroup, even if arising from a very limited sample size (Supplemental Figure 4 and Table 2). We observed lower absolute sensitivity rates in the earlier stages,[26] however, showing similar pooled diagnostic values compared to the advanced setting (Table 2).

Sample size. According to the median number of included patients (45 individuals), 12 and 13 studies were collected in the low- and high-size subgroups, showing the highest ctDNA performance in low-size studies according to the diagnostic values (Supplemental Figure 7a and b). Noteworthy, smaller studies added compelling insights in terms of pooled specificity and DOR

Table 2. Meta-analysis results.

	No of patients	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC
Overall	1966	0.73 (0.70–0.77)	0.87 (0.85–0.89)	7.94 (4.90–12.86)	0.33 (0.25–0.45)	33.41 (17.23–64.79)	0.93
Tumor burden							
Early	55	0.76 (0.57–0.90)	1.00 (0.87–1.00)	8.47 (0.97–73.91)	0.21 (0.02–2.55)	45.17 (1.13–1810.10)	1.00
Advanced	1836	0.77 (0.73–0.80)	0.86 (0.84–0.88)	8.16 (4.98–13.37)	0.29 (0.22–0.39)	40.53 (20.32–80.82)	0.92
Sample size							
Low	274	0.78 (0.70–0.85)	0.96 (0.91–0.98)	10.6 (2.5–45.9)	0.27 (0.15–0.46)	48.4 (11.38–205.9)	0.90
High	1698	0.72 (0.68–0.75)	0.85 (0.83–0.87)	7.2 (4.2–12.3)	0.36 (0.25–0.51)	27.11 (12.75–57.6)	0.87
Diagnostic technique							
NGS	307	0.83 (0.75–0.89)	0.98 (0.94–0.99)	11.65 (5.43–24.99)	0.23 (0.09–0.62)	59.80 (14.29–250.23)	0.98
ddPCR/BEAMing	1485	0.74 (0.70–0.78)	0.84 (0.82–0.86)	6.63 (3.97–11.08)	0.31 (0.22–0.43)	28.84 (13.45–61.86)	0.92
PCR	174	0.51 (0.39–0.64)	0.96 (0.91–0.99)	9.30 (0.64–136.17)	0.54 (0.31–0.96)	20.61 (1.57–270.46)	0.77
Sampling time							
Low-time	219	0.85 (0.75–0.92)	0.99 (0.96–1.00)	16.24 (6.23–42.31)	0.21 (0.1–0.47)	101.50 (23.22–443.62)	0.94
High-time	679	0.66 (0.59–0.73)	0.83 (0.80–0.87)	4.63 (2.46–8.73)	0.47 (0.31–0.70)	11.81 (5.15–27.10)	0.89
Biological subtype							
H+/HER2–	1357	0.73 (0.69–0.77)	0.83 (0.80–0.86)	5.97 (3.58–10.00)	0.32 (0.24–0.45)	22.94 (11.18–47.07)	0.87
HER2+	52	0.57 (0.35–0.77)	1.00 (0.88–1.00)	5.65 (1.69–18.95)	0.55 (0.37–0.82)	14.94 (3.00–74.54)	0.86
Hotspot mutation							
E542/545X	421	0.70 (0.58–0.81)	0.95 (0.92–0.97)	8.74 (3.47–22.02)	0.36 (0.16–0.82)	29.65 (7.55–116.41)	0.88
H1047X	520	0.74 (0.65–0.82)	0.98 (0.96–0.99)	18.57 (6.19–55.72)	0.30 (0.17–0.54)	83.38 (17.64–394.06)	0.93

AUC = area under the curve; BEAMing = beads, emulsions, amplification, and magnetics; CI, confidence interval; ddPCR = digital droplet polymerase chain reaction; DOR, diagnostic odds ratio HER2 = human epidermal growth factor receptor 2; HR = hormone receptor; NGS, next-generation sequencing; NLR, negative likelihood ratio; PLR, positive likelihood ratio.

compared to the heterogeneity of larger samples (0.96 and 40.42 *versus* 0.85 and 27.11, respectively) (Supplemental Figure 4).

Diagnostic technique. The most used techniques were ddPCR/BEAMing (12 cohorts, 1485 patients), followed by NGS (9 cohorts, 307 patients) and PCR (5 cohorts, 174 patients) (Supplemental Table 3). The ctDNA *PIK3CA* MAF was reported as the median and/or media of all mutated cases or calculated by extracting data from supplementary (7/25 studies) (Supplemental Table 7).[24,28,29,31,32,39,46] Namely, NGS seemed to outperform ddPCR/BEAMing and PCR in terms of sensitivity (0.83 *versus* 0.74 and 0.51, respectively) (Supplemental Figure 6 and Table 2). The ddPCR/BEAMing subgroup reported a lower pooled specificity (0.84) than NGS (0.98) and PCR (0.96). Furthermore, NGS outclassed PCR-based assays in terms of detection sensitivity, specificity, and AUC (0.98), not eventually leading to heterogeneity for specificity (Supplemental Figure 6a) while showing compelling PLR, NLR, and DOR rates that favored NGS over PCR-based methodologies (Table 2).

Sampling time. Among 20 studies, tissue biopsies were mainly performed on the primary site, with

four studies carrying out tissue biopsies on metastatic lesions (Supplemental Table 5). According to data available for 13 cohorts, the time between tissue and plasma sampling was variable, ranging from 0 day to over 15 years[23–26,29–31,35,39,43,44,46] (Supplemental Table 7d). Patients were assigned into low- and high-time subgroups, respectively (⩽ and >18 days), according to the median time between tissue and plasma collection. The best ctDNA performance in terms of sensitivity, specificity, and AUC (0.85, 0.99, and 0.94, respectively) was observed in the low-time subgroup, showing compelling findings for PLR, NLR, and DOR rates (16.24, 0.21, and 101.50, respectively) with acceptable heterogeneity (Supplemental Figure 7 and Table 2).

Biological subtype. The H+/HER2− and HER2+ subgroups were included in 5 and 10 studies (Supplemental Table 7)[25,32,34,36–38,40,44,46] with very few data being available on triple-negative BCs.[28,29,45] We found a comparable ctDNA performance for AUC (0.87 and 0.86, respectively) and other diagnostic rates, however observing higher ctDNA sensitivity favoring the H+/HER2− over the HER2+ subgroup (0.73 *versus* 0.57, respectively) (Supplemental Figure 7 and Table 2).

Hotspot mutation. Considering the most involved *PIK3CA* mutations within exons 9 and 20, 12 and 10 studies were pooled for the H1047X and E542/545X subgroups (520 and 421 patients, respectively) (Supplemental Table 4).[48–58] Specifically, ctDNA assays revealed a slightly more accurate trend in detecting H1047X than E542/545X in terms of sensitivity, specificity, and AUC (0.74, 0.98, and 0.93 versus 0.70, 0.95, and 0.88, respectively) (Supplemental Figure 7c–d and Table 2).

Discussion

In BC clinical practice, the tissue from primary lesions is typically available for diagnosis and biomarker testing in the basal setting. On the other hand, re-biopsies to obtain metastatic specimens of adequate quality and quantity may not always be feasible due to the location of the metastatic sites or patients' comorbidities. A growing body of evidence demonstrated that ctDNA represents a promising tool for predicting response to targeted treatment in solid tumors.[11,59] The choice of tumor tissue or liquid genotyping should be individualized in the clinical setting based on patient and disease characteristics, primarily considering that a reflex tumor tissue biopsy, if feasible, should be performed in the case of a ctDNA negative result to prevent FN results. With regard to BC, BELLE-2, BELLE-3, and SOLAR-1 were the first trials to include a survival analysis in ctDNA *PIK3CA*-positive patients. In this scenario, however, there is a lack of well-established data on sensitivity and specificity rates and concordance with tissue genotyping.

This individual patient data meta-analysis aimed to outline the diagnostic accuracy of ctDNA in evaluating the *PIK3CA* mutational status compared to tissue biopsy. Zhou *et al.*[60] have previously reported pooled optimal values of diagnostic performance of plasma ctDNA for prediction of *PIK3CA* mutation for sensitivity (0.86), specificity (0.98), AUC (0.99), PLR (42.8), and NLR (0.14). However, these results should be cautiously interpreted for the small sample size (247 patients from seven publications).[60] We found a highly accurate ctDNA performance in terms of sensitivity, specificity, and concordance with tissue testing from a larger sample size. The AUC curve supported these findings. Translating these overall pooled results in the clinic, the three-quarters of patients with a *PIK3CA*-positive tissue biopsy would test positive on ctDNA while only failing to be detected on plasma in the remaining cases. Furthermore, as shown by the NLR in Fagan's plot, a negative result of *PIK3CA* on plasma would lead to a three-fold decreased risk of finding a positive *PI3KCA* mutation on tissue. Nonetheless, the wide variability of the selected population in terms of several clinical, methodological, and technical conditions must be considered. While the meta-regression technique highlighted the sampling time as the main reason for heterogeneity, stratified subgroup analyses were performed to investigate the impact of specific variables on the diagnostic accuracy performance. Our meta-analysis, including more than 1800 patients with advanced *PIK3CA*-positive BC, provided a reliable estimation of the high ctDNA diagnostic accuracy in the metastatic setting, showing an AUC > 0.9, which is considered very accurate in clinical practice. We observed that most patients presented with visceral involvement and at least two metastatic lesions, thus including those tumors shedding high enough

ctDNA that would eventually avoid FN results. However, albeit showing comparable diagnostic values in early-stage BC, the controversial influence of *PIK3CA* mutations on survival outcomes in this subset of patients should be considered. In this regard, the scarce sample size (55 patients) along with the lower sensitivity rates critically affected the clinical utility of ctDNA which is to date already limited in early-stage BC, requiring further studies in the adjuvant setting before drawing any conclusions.

Considering the molecular diagnostic techniques, these pooled results consistently favored NGS over PCR-based methodologies. Overall, we found that NGS panels covered a broader spectrum of *PI3KCA* mutations, far beyond the FDA-approved detection of 11 activating mutations. These results were consistent with the exploratory analysis of the SOLAR-1 trial, revealing the ability of NGS testing to detect 60 different mutations across multiple exons and select *PI3KCA*-mutated patients who also benefited from alpelisib.[61,62] Considering the FDA-approved therascreen® RGQ PCR Kit (QIAGEN GmbH) ability to detect only hotspot mutations across three exons together with the eventual risk of generating FP results as highlighted by the ongoing market surveillance process, these findings would support the implementation of broader NGS panels either on tissue or plasma to screen for uncommon *PIK3CA* activating mutations that, however, remain to be further validated in clinical trials. Regarding the sampling time, remarkably, identifying a ctDNA *PIK3CA* mutation within 18 days from the tissue sampling would suggest a highly accurate concordance with histological genotyping, supporting the reliable use of a plasma-first approach that would likely allow overcoming the issue of intra-tumor heterogeneity. Referring to biological subtypes and common *PIK3CA* hotspot mutations, the ctDNA comparable performance between subgroups advised a similar impact on clinical decisions, even if the difference in both magnitude and different detection methods must be considered. Indeed, most of the patients were H+/Her2− and tested with PCR-based methodologies. Despite thoroughly encompassing all the publicly available data for detecting ctDNA *PIK3CA* mutations, some limitations of this meta-analysis should be considered. First, some of the included studies had missing data, affecting subgroup analyses. Second, our pooled results came from retrospective and prospective trials with different design conceptions that did neither aim to directly evaluate the prognostic/predictive role of *PI3KCA* mutations nor the correlation between the clearance of *PI3KCA* mutated allelic frequency and the radiologic response, although emerging data seemed to further validate the dynamic role of *PI3KCA* detected on ctDNA in the real-time longitudinal monitoring of BC.[63] Third, as partially discussed above, the heterogeneity of analyzed studies, including different disease stages and distribution, dissimilar sample sizes, the different prevalence of testing platforms, and timing for tissue and plasma sample collection, could have negatively affected the overall results. Notwithstanding, electronic databases, meeting proceedings, and other sources of gray literature research guarantee the systematicity of the literature review suggesting the high heterogeneity of the included studies is responsible for bias. Interestingly, subgroup analyses and meta-regression highlighted the sampling time as a possible cause of heterogeneity, reflecting the wide range between tissue and plasma sampling (0 and 15 years). Such heterogeneity should not affect the overall results, stating the ctDNA clinical utility for the *PIK3CA* mutational status evaluation.

In conclusion, these findings reliably estimate the ctDNA accuracy for detecting *PIK3CA* mutations, validating the role of liquid biopsy in the management of advanced BC. Considering the highest ctDNA accuracy in the metastatic setting, using highly sensitive NGS panels and when plasma is evaluated within 18 days from the tissue sampling, a ctDNA-first approach for the assessment of *PIK3CA* mutational status by NGS may accurately replace tissue tumor sampling, representing the preferable strategy at diagnosis of metastatic BC in patients who present with visceral involvement and at least two metastatic lesions, primarily given low clinical compliance or inaccessible metastatic sites (Figure 3). Larger clinical trials are warranted to further define the clinical utility of ctDNA accuracy for the detection of *PIK3CA* mutations in the early-stage BC setting.

Declarations

Ethics approval and consent to participate
Not Applicable.

Consent for publication
Not Applicable.

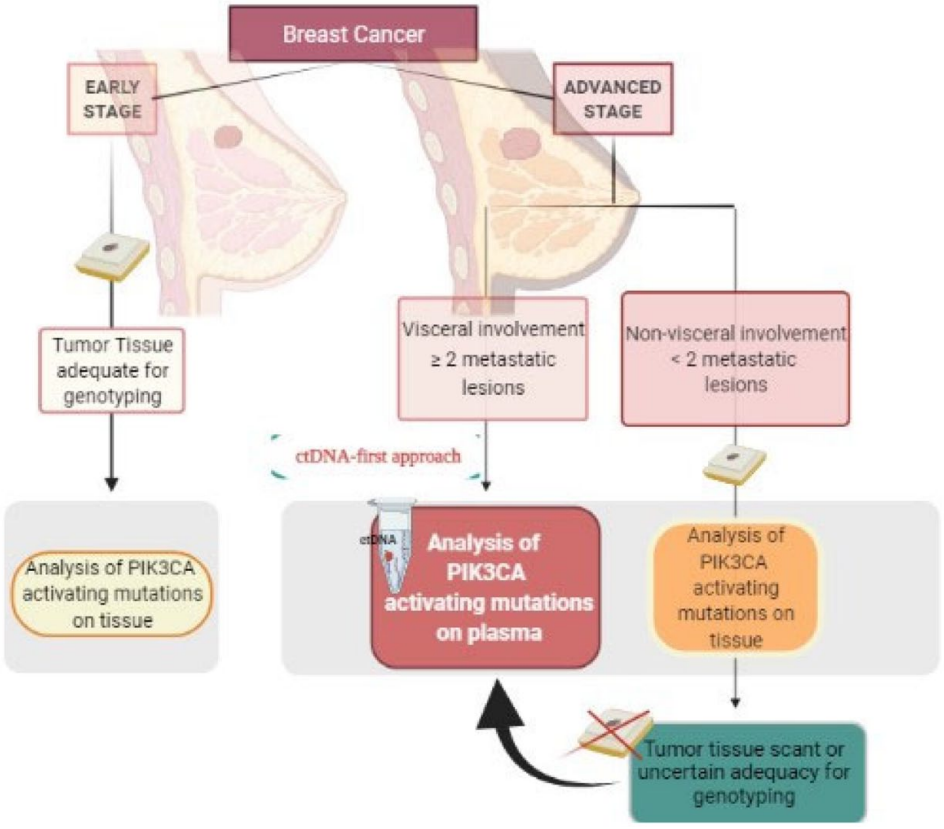

Figure 3. Algorithm depicting the role of ctDNA for the assessment of *PIK3CA* mutations in BC patients.
BC, breast cancer; ctDNA, circulating tumor DNA; *PIK3CA*, phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha.

Author contribution(s)

Antonio Galvano: Conceptualization; Data curation; Formal analysis; Methodology; Software; Supervision; Writing – review & editing.

Luisa Castellana: Conceptualization; Data curation; Formal analysis; Investigation; Writing – original draft.

Valerio Gristina: Data curation; Project administration; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

Maria La Mantia: Data curation; Visualization; Writing – review & editing.

Lavinia Insalaco: Validation.

Nadia Barraco: Resources; Supervision.

Alessandro Perez: Validation; Visualization.

Sofia Cutaia: Investigation.

Valentina Calò: Data curation.

Tancredi Didier Bazan Russo: Validation.

Edoardo Francini: Data curation; Project administration; Supervision; Validation; Visualization; Writing – review & editing.

Lorena Incorvaia: Resources.

Mario Giuseppe Mirisola: Validation; Visualization.

Salvatore Vieni: Validation; Visualization.

Christian Rolfo: Project administration; Resources; Software; Supervision; Validation; Visualization.

Viviana Bazan: Project administration; Resources; Software; Supervision; Validation; Visualization.

Antonio Russo: Project administration; Resources; Software; Supervision; Validation; Visualization.

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Competing interests

C.R. is a speaker for Merck Sharp and Dohme, AstraZeneca and has research collaborations with Guardant Health; advisory board activity: Archer, Inivata and MD Serono, Novartis, and BMS; non-financial support from Guardant Health; and research grant from LCRF-Pfizer. A.R. reported personal fees from Bristol, Pfizer, Bayer, Kyowa Kirin, Ambrosetti for advisory board activity; speaker honorarium from Roche Diagnostics. The remaining authors declare no potential conflicts of interest.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Supplemental material

Supplemental material for this article is available online.

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