

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

Alexandre Xu-Vuillard

`alexandre.xu-vuillard@gustaveroussy.fr`

Institut Gustave Roussy

Aurélia Robert

Institut Gustave Roussy

Barbara Pistilli

Institut Gustave Roussy

Suzette Delaloge

Institut Gustave Roussy

Jean Zeghondy

Institut Gustave Roussy

Chayma Bousrih

Institut Gustave Roussy

Adina Sabau

Institut Gustave Roussy

Fernanda Mosele

Institut Gustave Roussy

Alessandro Viansone

Institut Gustave Roussy

Elie Rassy

Institut Gustave Roussy

Thomas Grinda

Institut Gustave Roussy

Joana Mourato-Ribeiro

Institut Gustave Roussy

Cyril Roussel-Simonin

Institut Gustave Roussy

Keywords:**Posted Date:** October 18th, 2026**DOI:** <https://doi.org/10.21203/rs.3.rs-11053506/v1>**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.[Read Full License](#)

Additional Declarations: Competing interest reported. S.D. has received grants and non-financial support from Pfizer and AstraZeneca, and grants from Novartis, Roche Genentech, Lilly, Orion, Amgen, Sanofi, Exact Sciences, Servier, MSD, BMS, Pierre Fabre, Besins, the European Commission, the French government, Fondation ARC, Taiho and Elsan, outside the submitted work. B.P. has served in a consulting or advisory role for Puma Biotechnology, Novartis, Myriad Genetics and Pierre Fabre, has received personal fees from Novartis, AstraZeneca, MSD Oncology and Pfizer, and has received research funding from Daiichi Sankyo, Puma Biotechnology, Novartis, Merus, Pfizer and AstraZeneca. E.R. has received institutional research support from Gilead, MSD and Menarini, honoraria from Eli Lilly, Seagen, Novartis, AstraZeneca, Daiichi Sankyo, MSD, Menarini and Roche, and travel, accommodation and expenses support from Pfizer, Roche, Eli Lilly, Gilead, Novartis, Menarini, MSD and Mundipharma, and has served as a consultant for AstraZeneca, Novartis and MSD and on an advisory board for Gilead. J.M.-R. has received personal fees for advisory board participation from Gilead Sciences and as an invited speaker for eESO, and institutional fees for advisory board participation from AstraZeneca and Daiichi Sankyo, as an invited speaker for Roche-Genentech and as coordinating principal investigator for AstraZeneca and Roche-Genentech. F.M. has received consultant fees from Novartis and Pegascy. T.G. has received travel support from AstraZeneca, Gilead and Pfizer, has served in a consulting or advisory role for AstraZeneca, Pfizer, MSD and Cancérologie-Pratique, has received research funding from Amgen and has received a personal grant from the Philippe Foundation. A.V. has received honoraria from Seagen, has served in a consulting or advisory role for Seagen and on the speakers' bureau for AstraZeneca and Daiichi Sankyo, has received research funding from Pfizer, has provided expert testimony for Seagen and has received travel, accommodation and expenses support from Eisai Europe. C.B. has received travel, accommodation and expenses support from Roche, Novartis, Lilly, Amgen and Pfizer. J.Z. has received an institutional research grant from Menarini, travel and expenses support from Novartis, Eli Lilly, Pfizer, MSD and Roche, and institutional honoraria from Eli Lilly and Ipsen. All other authors (A.X.-V., A.R., A.S. and C.R.-S.) declare no financial or non-financial competing interests.

Abstract

Comprehensive circulating tumor DNA (ctDNA) profiling is often repeated in advanced breast cancer, yet its yield after a first informative assay is unknown. We analyzed 152 patients from the prospective STING trial (NCT04932525) with at least two FoundationOne Liquid CDx assays, classifying alterations with the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT). The first assay was contributive in 87 patients (57.2%), and 46 of 65 non-contributive first assays (70.8%) later became contributive. Gene-level concordance between consecutive contributive assays was high (median Matthews correlation coefficient 0.86; $n = 75$). Newly emergent ESCAT alterations other than *ESR1* mutations occurred in 6 of 96 patients (6.3%) and were predominantly subclonal (7 of 8 evaluable variants); 6 patients (6.3%) acquired an *ESR1* mutation. Only 9 of 49 matched treatment opportunities (18.4%) arose from re-profiling. Repeat comprehensive profiling is informative after a non-contributive assay, whereas targeted *ESR1* monitoring is preferable after a contributive one.

Clinical trial registration: ClinicalTrials.gov, NCT04932525, registered on 1 June 2021.

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among women worldwide¹, and progression to advanced disease marks a turning point associated with markedly shorter survival². Treatment selection in advanced breast cancer (ABC) increasingly depends on actionable molecular alterations. The phosphatidylinositol 3-kinase α (PI3K α) inhibitors alpelisib³ and inavolisib⁴ prolong progression-free survival when combined with endocrine therapy in *PIK3CA*-mutated, hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) disease, and poly(ADP-ribose) polymerase (PARP) inhibitors such as olaparib⁵ and talazoparib⁶ confer clinically meaningful benefit in patients with germline *BRCA1* or *BRCA2* mutations, anchoring molecular profiling in routine practice⁷.

Circulating tumor DNA (ctDNA) analysis has become a key tool for tumor molecular profiling⁸. Plasma assays detect alterations ranked by the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT)⁹, facilitate access to biomarker-driven trials and identify targets for emerging therapies, including mutant-selective AKT1 inhibitors (ALTA2618, NCT06533059), novel HER2-directed tyrosine kinase inhibitors^{10–12} and antibody-drug conjugates¹³. Liquid biopsy is also central to the detection of *ESR1* mutations, a major mechanism of acquired resistance to aromatase inhibitors in HR+/HER2 - ABC¹⁴ that can be targeted with oral selective estrogen receptor degraders (SERDs)^{15,16}. The PADA-1¹⁷ and SERENA-6¹⁸ trials showed that detecting emerging *ESR1* mutations by serial ctDNA testing and switching treatment before radiological progression prolongs progression-free survival.

Despite these advances, no evidence-based recommendation defines the indication, frequency or clinical utility of repeat comprehensive ctDNA profiling. As broad panels are increasingly ordered at several

timepoints, clinicians must judge whether newly reported alterations reflect true clonal evolution or subclonal events of uncertain significance¹⁹. The stakes are practical: subclonal alterations may be misread as actionable targets and prompt treatment changes unsupported by evidence, whereas repeated comprehensive testing incurs substantial costs of unproven benefit.

Here, we used serial plasma samples from the prospective STING trial to determine whether repeat comprehensive liquid biopsy yields actionable genomic information beyond the first informative assay in patients with ABC.

Results

Patient characteristics and liquid biopsy sampling

We included 152 patients with ABC who underwent at least two comprehensive ctDNA assays (Table 1). Most had HR+/HER2– disease (123 of 152, 80.9%), followed by triple-negative breast cancer (TNBC; 25, 16.4%) and HER2-positive disease (4, 2.6%). Median age at the first liquid biopsy (LB1) was 53.9 years (interquartile range (IQR) 46.9-62.6). LB1 was performed before any line of therapy for advanced disease in 58 patients (38.2%), after one line in 35 (23.0%) and after two or more lines in 59 (38.8%). Patients underwent a median of 2 liquid biopsies (range 2-6): 128 (84.2%) had two, 21 (13.8%) three and 3 (2.0%) four or more. The median interval between LB1 and the second liquid biopsy (LB2) was 12.7 months (IQR 6.7-21.7).

Table 1 | Baseline characteristics of the study population (N = 152)

Characteristic	Unit	N available	Overall population (N = 152)
Demographics			
Age at LB1	median, years (IQR)	152	53.9 (46.9-62.6)
Histology and phenotype			
Invasive ductal carcinoma	n (%)	152	142/152 (93.4%)
Invasive lobular carcinoma	n (%)	152	10/152 (6.6%)
HR+/HER2-	n (%)	152	123/152 (80.9%)
Triple-negative breast cancer	n (%)	152	25/152 (16.4%)
HER2-positive	n (%)	152	4/152 (2.6%)
Metastatic setting			
De novo metastatic disease	n (%)	151	50/151 (33.1%)
Metastatic relapse	n (%)	151	101/151 (66.9%)
Time from metastatic diagnosis to LB1	median, months (IQR)	151	13.4 (3.6-42.7)
Prior therapy before LB1			
Number of prior treatment lines	median (IQR)	152	1 (0-3)
No prior line (first metastatic line)	n (%)	152	58/152 (38.2%)
One prior line	n (%)	152	35/152 (23.0%)
≥2 prior lines	n (%)	152	59/152 (38.8%)
Extent of metastatic disease at LB1			
Number of metastatic sites	median (IQR)	151	2 (1-3)
Bone metastases	n (%)	151	91/151 (60.3%)
Bone-only disease	n (%)	151	32/151 (21.2%)
Liver metastases	n (%)	151	51/151 (33.8%)
Visceral disease (any site)	n (%)	151	68/151 (45.0%)
Lung and/or pleural metastases	n (%)	148	26/148 (17.6%)
Lymph node metastases	n (%)	151	87/151 (57.6%)
Central nervous system metastases	n (%)	151	5/151 (3.3%)

Characteristic	Unit	N available	Overall population (N = 152)
Skin and/or chest wall metastases	n (%)	151	15/151 (9.9%)
Laboratory parameters at LB1			
LDH	median, U/L (IQR)	77	212 (186-264)
Elevated LDH (>250 U/L)	n (%)	77	22/77 (28.6%)
Liquid biopsy			
Contributive LB1 (ctDNA detected)	n (%)	152	87/152 (57.2%)
Non-contributive LB1	n (%)	152	65/152 (42.8%)
Total number of liquid biopsies	median (IQR)	152	2 (2-2)

Abbreviations: ctDNA, circulating tumor DNA; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IQR, interquartile range; LB1, first liquid biopsy; LDH, lactate dehydrogenase; U/L, units per liter. Percentages are calculated among patients with available data.

Most non-contributive first assays become informative on repeat sampling

LB1 was contributive in 87 patients (57.2%) and non-contributive in 65 (42.8%) (Fig. 1). Of these 65 patients, 42 (64.6%) converted to contributive status at LB2, 3 (4.6%) at LB3 and 1 (1.5%) at LB4, an overall conversion rate of 70.8% (46 of 65), with a median time to conversion of 14.8 months (IQR 11.5-22.5). Overall, 133 patients (87.5%) had at least one contributive liquid biopsy, 96 had two or more, and 19 (12.5%) remained non-contributive at all available timepoints (Fig. 1).

Patients with a contributive LB1 more frequently had liver metastases (41.9% versus 22.2%; $P = 0.021$) and less frequently bone-only disease (10.8% versus 31.5%; $P = 0.004$) than those with a non-contributive LB1. Age ($P = 0.78$), number of prior treatment lines ($P = 0.41$), number of metastatic sites ($P = 0.10$), visceral disease ($P = 0.11$) and central nervous system metastases ($P = 0.08$) did not differ substantially between groups.

The 19 persistently non-contributive patients were predominantly HR+/HER2- (18 of 19, 94.7%), and most had undergone only two liquid biopsies (15 of 19, 78.9%). In univariable analysis, bone-only disease was the only factor associated with persistent non-contributivity (9 of 19, 47.4%, versus 23 of 132, 17.4%, of patients with at least one contributive biopsy; $P = 0.006$); no association was found with age, prior treatment lines, visceral or liver metastases, subtype or lactate dehydrogenase level. Among the 46 patients who converted, the timing of conversion was not associated with any baseline clinical characteristic.

Actionable alterations are frequent at the first contributive assay

Of the 133 patients with at least one contributive biopsy, 33 with hypermutated tumors (tumor mutational burden (TMB) >10 mutations per megabase) were excluded from the landscape, concordance and clonality analyses (Methods). Of the remaining 100 patients, 73 (73.0%) harbored at least one ESCAT tier I-III alteration, most frequently in *PIK3CA* (35 patients, 35.0%), *ESR1* (24, 24.0%), *BRCA2* (9, 9.0%) and *ARID1A* (9, 9.0%) (Fig. 2). Tier I (*PIK3CA*, *ESR1*, *BRCA1* and *BRCA2*) and tier IIIA (*KRAS*, *ARID1A* and *BRAF*) alterations predominated. *PIK3CA* (42%) and *ESR1* (31%) alterations were the most frequent in HR+/HER2- disease (n = 77), and *BRCA2* (13%), *KRAS* (9%) and *BRCA1* (9%) alterations in TNBC (n = 23).

The actionable genomic landscape is stable across serial assays

The number of mutations per patient did not change substantially between the first contributive biopsy (LB_n) and the next contributive biopsy (LB_{n+1}) (median 3 versus 3; *P* = 0.074; Fig. 3a). It increased in 54.8% of patients, mostly through new variants in genes associated with clonal hematopoiesis (*DNMT3A*, *TET2*, *ASXL1* and *TP53*)²⁰ (Fig. 3b). The number of ESCAT-classified mutations was likewise unchanged between LB_n and LB_{n+1} (median 1 versus 1; *P* = 0.12) and between LB_{n+1} and LB_{n+2} (*P* = 1.0).

Gene-level concordance between consecutive contributive biopsies was assessed in 75 patients with non-hypermutated tumors. Concordance was high across the 11 ESCAT-classified genes evaluated (median Matthews correlation coefficient (MCC) 0.86): seven genes reached an MCC of 0.80 or more (*IDH2*, *AKT1*, *PIK3CA*, *BRCA2*, *ARID1A*, *FGFR2* and *BRCA1*), concordance was lower for *KRAS* (0.76) and *ESR1* (0.75), and it was low for two infrequently altered genes, *ERBB2* (0.49) and *BRAF* (-0.03) (Fig. 4a). Concordance of ESCAT tier I-IIIa alterations was perfect in TNBC (MCC 1.0; Fig. 4b). Cohen's kappa and MCC ranked genes identically (Spearman's ρ = 1.00; *P* < 0.001).

Newly emergent actionable alterations are rare and mostly subclonal

Among the 96 patients with at least two contributive biopsies, seven newly emergent ESCAT-classified alterations other than *ESR1* mutations were detected in 6 patients (6.3%; Fig. 5a): two *BRAF* alterations (including p.V600E), *PIK3CA* p.E545K, an *ERBB2* exon 19 deletion, *KRAS* p.Q61H, and one alteration each in *KIT* and *MTAP*; one patient acquired both the *KRAS* and a *BRAF* alteration. All occurred at the LB_n to LB_{n+1} transition; no new ESCAT-classified alteration was detected at LB_{n+2} in patients with three consecutive contributive biopsies. Six additional patients (6.3%) acquired an *ESR1* mutation (Fig. 5a).

Most newly detected alterations had a low variant allele frequency (VAF) relative to tumor fraction (TF), suggesting subclonal origin (Supplementary Fig. 1). For instance, the emergent *PIK3CA* p.E545K mutation was detected at a VAF of 0.14% despite a TF of 48.0% and co-occurring clonal *GATA3* and *ARID1A* alterations, which questions its actionability (Supplementary Fig. 1b). Overall, clonality could be assessed for 8 of 15 newly detected ESCAT-classified variants, TF being unavailable for 7 *ESR1* variants; 7 of these 8 variants (87.5%) were subclonal, the only clonal one being the *KRAS* p.Q61H mutation (VAF 7.56%) (Supplementary Fig. 1a).

Re-profiling accounts for a minority of matched treatment opportunities

Forty-nine of the 96 patients with at least two contributive biopsies (51.0%) accessed a molecularly matched clinical trial or early access program: 34 on the basis of alterations identified at LB1, 9 on the basis of mutations newly detected at a subsequent biopsy and 6 through repeat biopsies beyond LB n +1, none of which was driven by a newly emergent mutation. Only 9 of the 49 matched opportunities (18.4%) therefore rested on an alteration first detected at re-profiling.

Discussion

In this analysis of serial comprehensive ctDNA profiling in ABC, the actionable genomic landscape defined by the first contributive assay remained stable over time. Gene-level concordance of ESCAT-classified alterations was high, newly emergent ESCAT alterations other than *ESR1* mutations were uncommon, and most newly detected variants were either attributable to clonal hematopoiesis or present at low VAF relative to TF, pointing to a subclonal origin and uncertain therapeutic relevance. Consistently, fewer than one in five matched treatment opportunities arose from re-profiling. Once a contributive comprehensive profile has been obtained, systematically repeating the same broad-panel assay is therefore unlikely to substantially increase the yield of actionable targets.

This conclusion does not extend to patients whose first assay is non-contributive. Seven in ten of these patients became ctDNA-positive at a later timepoint, enabling a genomic characterization that was impossible at baseline and, in some cases, revealing actionable alterations. A non-contributive plasma assay should therefore be read as the absence of detectable tumor-derived DNA at that timepoint, not as evidence that the tumor lacks actionable alterations. ctDNA detectability varies with tumor burden, disease kinetics, metastatic distribution, treatment exposure and intrinsic shedding, and the association between persistent non-contributivity and bone-only disease in our cohort is consistent with low shedding from bone-predominant disease. Repeating comprehensive profiling at a clinically relevant later timepoint, particularly at progression, is thus justified after a non-contributive assay.

Our findings do not question the value of longitudinal ctDNA assessment as such; rather, they distinguish comprehensive profiling for target identification from targeted monitoring of dynamic predictive biomarkers. *ESR1* is the clearest example of the latter. *ESR1* mutations emerge under the selective pressure of aromatase inhibitors and are a well-established mechanism of acquired endocrine resistance²¹, and the PADA-1¹⁷ and SERENA-6¹⁸ trials showed that ctDNA-guided pre-emptive treatment switching prolongs progression-free survival. Given the need for repeated testing and the frequency of this alteration, sensitive and inexpensive targeted assays are better suited than repeat comprehensive profiling: within PADA-1, droplet digital PCR (ddPCR) enabled serial *ESR1* monitoring across more than 12,500 blood samples, with cross-validation against next-generation sequencing (NGS)²².

Questioning the value of repeated comprehensive profiling is not an argument against broad-panel sequencing at the first liquid biopsy. In the randomized PRIME trial, a 324-gene panel identified

molecularly based recommended therapies in 51.6% of patients with advanced solid tumors versus 36.9% with an 87-gene panel, with nearly twice as many actionable alterations in the breast cancer subgroup²³. The modest benefit observed in matched-therapy trials likely reflects limited treatment access, drug efficacy and disease stage at intervention rather than an intrinsic failure of broad profiling, and oncology is moving towards a tumor-agnostic paradigm in which genomic alterations define eligibility irrespective of histology²⁴. Maximizing the breadth of initial profiling therefore remains desirable, whereas repeat comprehensive profiling should be reserved for selected situations.

Building on these results and on evidence from the SAFIR02-BREAST and SAFIR-PI3K trials that matched targeted therapies prolong progression-free survival only in patients with ESCAT tier I or II alterations²⁵, we propose a pragmatic framework towards the rational use of serial ctDNA profiling (Fig. 6). After a contributive first assay, routine repeat comprehensive profiling appears of limited value; it may be considered when a clinical trial becomes available for a previously unassessed target or when the disease phenotype changes substantially, for example with the development of brain metastases or histological transformation. In patients with HR+/HER2- disease receiving an aromatase inhibitor and with access to a SERD, targeted *ESR1* testing by ddPCR or a focused NGS panel should be performed after at least 6 months of treatment or at progression. After a non-contributive first assay, comprehensive profiling should be repeated at progression or at the next treatment line; if it remains non-contributive despite documented progression, tissue re-biopsy should be considered where feasible. Because our cohort is monocentric, these proposals are not guidelines and require prospective validation in larger multicenter cohorts.

This study has limitations. First, the timing of repeat liquid biopsies was left to the treating physician, which may have selected patients with more complex disease trajectories. Second, the cohort was predominantly HR+/HER2-, which limits generalizability to HER2-positive and triple-negative disease. Third, tissue was not re-biopsied at matched timepoints, so we cannot exclude that ctDNA under-represents spatially heterogeneous or low-shedding subclones. Fourth, the VAF/(TF/2) approximation of clonality does not account for local copy-number changes or tumor aneuploidy. Finally, all analyses relied on a single assay, and generalizability to other platforms requires validation. Nonetheless, the mutational landscape at the index biopsy, dominated by *PIK3CA*, *ESR1*, *BRCA2* and *ARID1A* alterations, matches that reported in other cohorts²⁶⁻²⁸, supporting the representativeness of our population.

In summary, the indication for serial liquid biopsy in ABC should depend on the result of the first assay. Repeat comprehensive profiling is informative after a non-contributive assay but adds little after a contributive one, for which targeted *ESR1* monitoring under endocrine therapy is the principal exception. A selective, biology-driven strategy would concentrate comprehensive genomic profiling where it is most likely to change patient care.

Methods

Study design and participants

This is a secondary analysis of the prospective STING trial (NCT04932525), conducted at Gustave Roussy (Villejuif, France) to evaluate the clinical utility of ctDNA analysis across solid tumors; results were reviewed at weekly multidisciplinary molecular tumor boards. Eligible patients were aged 18 years or older with histologically confirmed metastatic or locally advanced breast cancer. Blood was sampled at enrollment, which could occur at any point of the advanced disease course: before treatment initiation, during systemic therapy or at progression. Patients concurrently enrolled in other interventional trials were excluded. The present analysis included all patients with at least two ctDNA assessments. The timing of repeat sampling was left to the treating physician, typically at radiological progression, at the start of a new treatment line or when a new therapeutic opportunity arose.

Ethics

The STING trial (NCT04932525; ID-RCB 2020-A03547-32), sponsored by Gustave Roussy, was approved by a French Comité de Protection des Personnes (CPP) and by the French National Agency for Medicines and Health Products Safety (ANSM), and was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before enrollment and blood collection. Clinical trial registration: ClinicalTrials.gov, NCT04932525, registered on 1 June 2021.

ctDNA sequencing

Plasma ctDNA was sequenced with the FoundationOne Liquid CDx assay (Foundation Medicine, Cambridge, MA, USA), a hybrid capture-based NGS assay that interrogates up to 324 genes depending on panel version (Supplementary Data 1) for single-nucleotide variants, short insertions and deletions, copy-number alterations and selected rearrangements. According to the manufacturer's technical specifications, the median limit of detection for short variants is 0.40% VAF for genes captured with enhanced sensitivity and 0.82% VAF for the other genes (Supplementary Table 1).

Definition of contributive liquid biopsies

A liquid biopsy was considered contributive when at least one somatic alteration confidently attributed to tumor-derived ctDNA was detected, and non-contributive when no tumor-derived variant was detected or when all detected variants were compatible with clonal hematopoiesis.

ESCAT classification

All alterations were classified according to ESCAT⁹. Tier I denotes targets ready for implementation in routine clinical decisions: tier IA, an alteration-drug match associated with improved survival in prospective randomized trials in the same tumor type; tier IB, clinically meaningful benefit in prospective non-randomized trials in the same tumor type; tier IC, benefit in basket trials or trials across tumor types. Tier II denotes investigational targets likely to define a population that benefits from a targeted drug but for which additional data are needed: tier IIA, greater benefit in patients with the alteration in retrospective analyses of randomized trials; tier IIB, increased responsiveness to the matched drug in prospective trials, without survival data. Tier III denotes alterations for which clinical benefit has been

shown in another tumor type (IIIA), or alterations not yet studied in clinical trials but with a predicted functional impact similar to that of a tier I alteration in the same gene (IIIB). Tier IV denotes preclinical evidence of actionability, from in vitro or in vivo models (IVA) or from in silico prediction (IVB). Tier X denotes the absence of evidence for actionability.

Longitudinal analysis

The first contributive liquid biopsy of each patient was designated LB_n , irrespective of its rank (LB_1 to LB_4), and the next contributive sample LB_{n+1} , so that genomic evolution could be analyzed independently of biopsy number. All consecutive contributive pairs were analyzed. A mutation was considered newly emergent at LB_{n+1} when the gene had not been altered in any earlier contributive biopsy from the same patient; this definition avoids misclassifying intermittently detected variants as new. Patients with a TMB >10 mutations per megabase at the first contributive biopsy were excluded from the landscape, concordance and clonality analyses, because a markedly elevated background mutation rate precludes assigning clinical significance to individual ESCAT-classified alterations.

Study objectives

The primary objective was the proportion of patients with newly emergent actionable (ESCAT tier I-III) mutations at subsequent liquid biopsies relative to the first contributive assay. Secondary objectives were genomic concordance between serial biopsies, the nature of newly emergent alterations, their clonal or subclonal status, the impact of serial liquid biopsy on therapeutic decisions and clinical factors associated with non-contributive biopsies. The exploratory objective was to propose a framework for the rational use of repeat testing.

Concordance analysis

For each ESCAT-classified gene, the presence or absence of an alteration at LB_n and LB_{n+1} was cross-tabulated across patients, and concordance was quantified with the MCC:

$$MCC = (TP \times TN - FP \times FN) / \sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}$$

where TP and TN are the numbers of patients in whom the alteration was detected at both or at neither timepoint, and FP and FN the numbers in whom it was detected only at LB_{n+1} or only at LB_n , respectively. The MCC ranges from -1 to 1 and, because it incorporates all four cells of the contingency table, remains informative under the class imbalance of this setting, in which most gene-patient observations are negative at both timepoints²⁹. Cohen's kappa was computed on the same tables as a sensitivity analysis.

Clonality inference

Clonality was inferred from the ratio $VAF/(TF/2)$, a clonal heterozygous mutation in a diploid tumor being expected at a VAF of approximately $TF/2$ ³⁰⁻³². Mutations were classified as clonal when this ratio was

≥0.5 and as subclonal otherwise.

Statistics and reproducibility

Continuous variables are summarized as medians with IQR and categorical variables as counts and percentages, with the denominator of each analysis stated in the text and figure legends. Paired mutation counts at LB n and LB $n+1$ were compared with the two-sided Wilcoxon signed-rank test, chosen because counts are discrete and not normally distributed. The agreement between MCC and Cohen's kappa across genes was assessed with Spearman's rank correlation. Clinical characteristics were compared between groups with Pearson's χ^2 test, or Fisher's exact test when an expected cell count was below 5, in univariable analyses; no multivariable model was fitted given the small number of events. Time to conversion was measured from LB1 to the first contributive liquid biopsy. All tests were two-sided with an alpha level of 0.05. No adjustment was made for multiple comparisons because these analyses were exploratory, and P values should be interpreted accordingly. Analyses were performed in R version 4.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Declarations

Competing Interests

S.D. has received grants and non-financial support from Pfizer and AstraZeneca, and grants from Novartis, Roche Genentech, Lilly, Orion, Amgen, Sanofi, Exact Sciences, Servier, MSD, BMS, Pierre Fabre, Besins, the European Commission, the French government, Fondation ARC, Taiho and Elsan, outside the submitted work. B.P. has served in a consulting or advisory role for Puma Biotechnology, Novartis, Myriad Genetics and Pierre Fabre, has received personal fees from Novartis, AstraZeneca, MSD Oncology and Pfizer, and has received research funding from Daiichi Sankyo, Puma Biotechnology, Novartis, Merus, Pfizer and AstraZeneca. E.R. has received institutional research support from Gilead, MSD and Menarini, honoraria from Eli Lilly, Seagen, Novartis, AstraZeneca, Daiichi Sankyo, MSD, Menarini and Roche, and travel, accommodation and expenses support from Pfizer, Roche, Eli Lilly, Gilead, Novartis, Menarini, MSD and Mundipharma, and has served as a consultant for AstraZeneca, Novartis and MSD and on an advisory board for Gilead. J.M.-R. has received personal fees for advisory board participation from Gilead Sciences and as an invited speaker for eESO, and institutional fees for advisory board participation from AstraZeneca and Daiichi Sankyo, as an invited speaker for Roche-Genentech and as coordinating principal investigator for AstraZeneca and Roche-Genentech. F.M. has received consultant fees from Novartis and Pegascy. T.G. has received travel support from AstraZeneca, Gilead and Pfizer, has served in a consulting or advisory role for AstraZeneca, Pfizer, MSD and Cancérologie-Pratique, has received research funding from Amgen and has received a personal grant from the Philippe

Foundation. A.V. has received honoraria from Seagen, has served in a consulting or advisory role for Seagen and on the speakers' bureau for AstraZeneca and Daiichi Sankyo, has received research funding from Pfizer, has provided expert testimony for Seagen and has received travel, accommodation and expenses support from Eisai Europe. C.B. has received travel, accommodation and expenses support from Roche, Novartis, Lilly, Amgen and Pfizer. J.Z. has received an institutional research grant from Menarini, travel and expenses support from Novartis, Eli Lilly, Pfizer, MSD and Roche, and institutional honoraria from Eli Lilly and Ipsen. All other authors (A.X.-V., A.R., A.S. and C.R.-S.) declare no financial or non-financial competing interests.

Author Contribution

A.X.-V. and A.R. contributed equally. A.X.-V., A.R. and C.R.-S. conceived the study. C.R.-S. curated and analyzed the data. A.X.-V., A.R. and C.R.-S. developed the methodology. C.R.-S. prepared the figures. B.P., S.D., J.Z., C.B., A.S., A.V., E.R., J.M.-R. and A.R. enrolled patients and collected clinical data. C.R.-S. supervised the study. A.X.-V. and A.R. wrote the first draft of the manuscript. All authors reviewed and edited the manuscript, and read and approved the final version.

Acknowledgement

We thank the patients and their families, and the teams of the Breast Cancer Department and the Precision Medicine program at Gustave Roussy. The STING trial is sponsored by Gustave Roussy. This analysis received no specific funding.

Data Availability

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

Code availability

The R code used for the analyses is not publicly available but is available from the corresponding author on reasonable request.

References

1. Bray, F. et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **74**, 229-263 (2024).
2. Grinda, T. et al. Evolution of overall survival and receipt of new therapies by subtype among 20 446 metastatic breast cancer patients in the 2008-2017 ESME cohort. *ESMO Open* **6**, 100114 (2021).

3. André, F. et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. *N. Engl. J. Med.* **380**, 1929-1940 (2019).
4. Turner, N. C. et al. Inavolisib-based therapy in PIK3CA-mutated advanced breast cancer. *N. Engl. J. Med.* **391**, 1584-1596 (2024).
5. Robson, M. et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. *N. Engl. J. Med.* **377**, 523-533 (2017).
6. Litton, J. K. et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. *N. Engl. J. Med.* **379**, 753-763 (2018).
7. Gennari, A. et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. *Ann. Oncol.* **32**, 1475-1495 (2021).
8. Pascual, J. et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann. Oncol.* **33**, 750-768 (2022).
9. Mateo, J. et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). *Ann. Oncol.* **29**, 1895-1902 (2018).
10. Nakamura, Y. et al. Tucatinib and trastuzumab for previously treated human epidermal growth factor receptor 2-positive metastatic biliary tract cancer (SGNTUC-019): a phase II basket study. *J. Clin. Oncol.* **41**, 5569-5578 (2023).
11. Le, X. et al. Sevabertinib in advanced HER2-mutant non-small-cell lung cancer. *N. Engl. J. Med.* **393**, 1819-1832 (2025).
12. Heymach, J. V. et al. Zongertinib in previously treated HER2-mutant non-small-cell lung cancer. *N. Engl. J. Med.* **392**, 2321-2333 (2025).
13. Li, B. T. et al. Trastuzumab deruxtecan in patients with solid tumours harbouring specific activating HER2 mutations (DESTINY-PanTumor01): an international, phase 2 study. *Lancet Oncol.* **25**, 707-719 (2024).
14. Cabel, L. et al. Kinetics and determinants of ESR1 mutation detection in metastatic breast cancer. *Ann. Oncol.* **37**, 329-340 (2026).
15. Bardia, A. et al. Elacestrant in ER+, HER2- metastatic breast cancer with ESR1-mutated tumors: subgroup analyses from the phase III EMERALD trial by prior duration of endocrine therapy plus CDK4/6 inhibitor and in clinical subgroups. *Clin. Cancer Res.* **30**, 4299-4309 (2024).
16. Jhaveri, K. L. et al. Imlunestrant with or without abemaciclib in advanced breast cancer. *N. Engl. J. Med.* **392**, 1189-1202 (2025).
17. Bidard, F.-C. et al. Switch to fulvestrant and palbociclib versus no switch in advanced breast cancer with rising ESR1 mutation during aromatase inhibitor and palbociclib therapy (PADA-1): a randomised, open-label, multicentre, phase 3 trial. *Lancet Oncol.* **23**, 1367-1377 (2022).

18. Bidard, F.-C. et al. First-line camizestrant for emerging ESR1-mutated advanced breast cancer. *N. Engl. J. Med.* **393**, 569-580 (2025).
19. Murtaza, M. et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* **497**, 108-112 (2013).
20. Magee, D. et al. Characterization of plasma cell-free DNA variants as of tumor or clonal hematopoiesis origin in 16,812 advanced cancer patients. *Clin. Cancer Res.* **31**, 2710-2718 (2025).
21. Jeselsohn, R., Buchwalter, G., De Angelis, C., Brown, M. & Schiff, R. ESR1 mutations—a mechanism for acquired endocrine resistance in breast cancer. *Nat. Rev. Clin. Oncol.* **12**, 573-583 (2015).
22. Callens, C. et al. Real-time detection of ESR1 mutation in blood by droplet digital PCR in the PADA-1 trial: feasibility and cross-validation with NGS. *Anal. Chem.* **94**, 6297-6303 (2022).
23. Trédan, O. et al. Broad versus limited gene panels to guide treatment in patients with advanced solid tumors: a randomized controlled trial. *Nat. Med.* **31**, 1502-1508 (2025).
24. Hyman, D. M., Taylor, B. S. & Baselga, J. Implementing genome-driven oncology. *Cell* **168**, 584-599 (2017).
25. André, F. et al. Genomics to select treatment for patients with metastatic breast cancer. *Nature* **610**, 343-348 (2022).
26. Fribbens, C. et al. Plasma ESR1 mutations and the treatment of estrogen receptor-positive advanced breast cancer. *J. Clin. Oncol.* **34**, 2961-2968 (2016).
27. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature* **490**, 61-70 (2012).
28. Bertucci, F. et al. Genomic characterization of metastatic breast cancers. *Nature* **569**, 560-564 (2019).
29. Chicco, D. & Jurman, G. The Matthews correlation coefficient (MCC) should replace the ROC AUC as the standard metric for assessing binary classification. *BioData Min.* **16**, 4 (2023).
30. Johnson, B. et al. Comprehensive landscape of BRAF variant classes, clonalities, and comutations in metastatic colorectal cancer using ctDNA profiling. *J. Clin. Oncol.* **40** (Suppl. 4), 152 (2022).
31. Morris, V. K. et al. Utility of circulating tumor DNA in the clinical management of patients with BRAF V600E metastatic colorectal cancer. *J. Clin. Oncol.* **39** (Suppl. 3), 119 (2021).
32. Chakravarty, D. et al. Somatic genomic testing in patients with metastatic or advanced cancer: ASCO provisional clinical opinion. *J. Clin. Oncol.* **40**, 1231-1258 (2022).

Figures

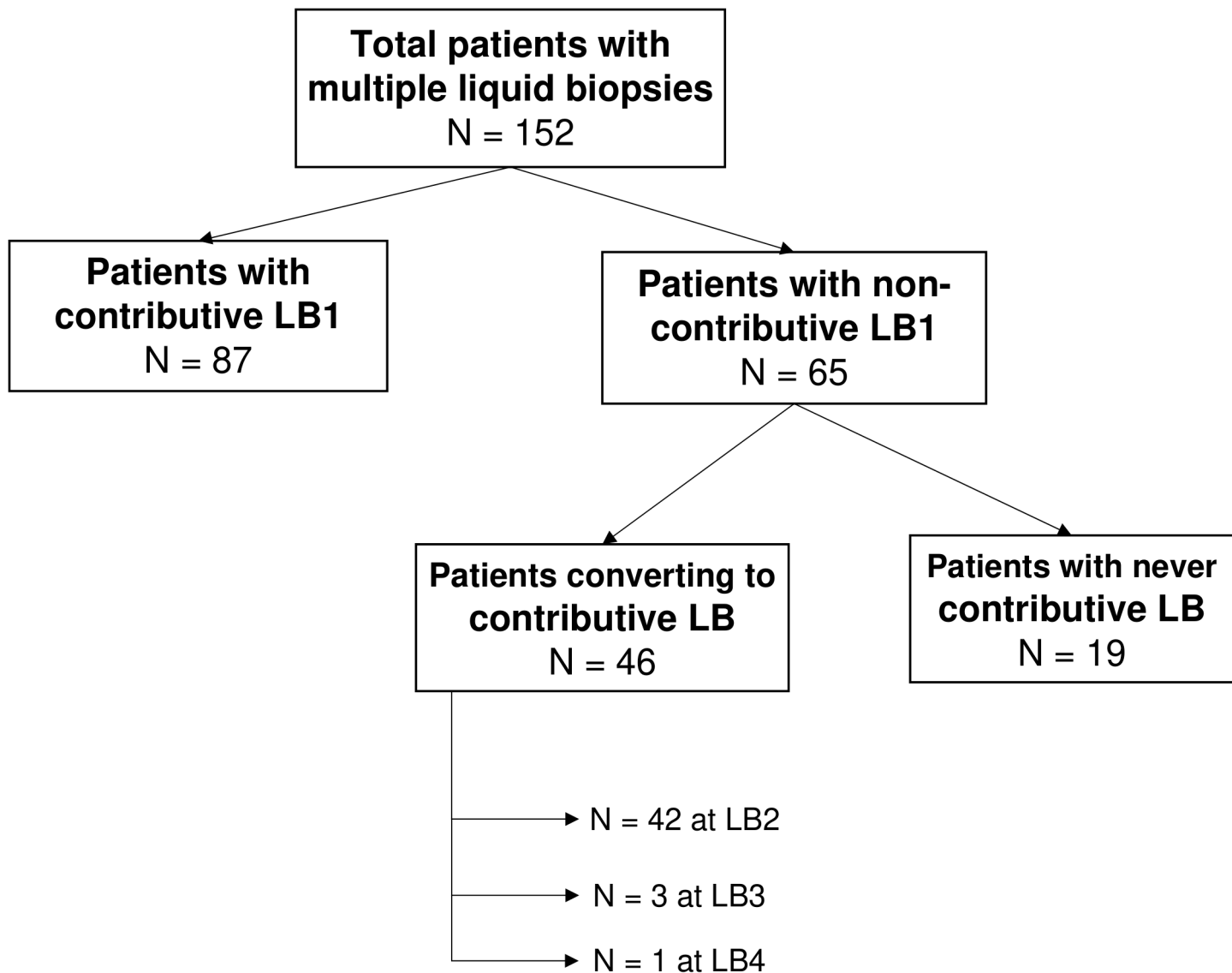


Figure 1

Study flow chart. Distribution of the 152 patients with advanced breast cancer according to the contributivity of the first liquid biopsy (LB1) and to conversion to contributive status at subsequent liquid biopsies (LB2 to LB4).

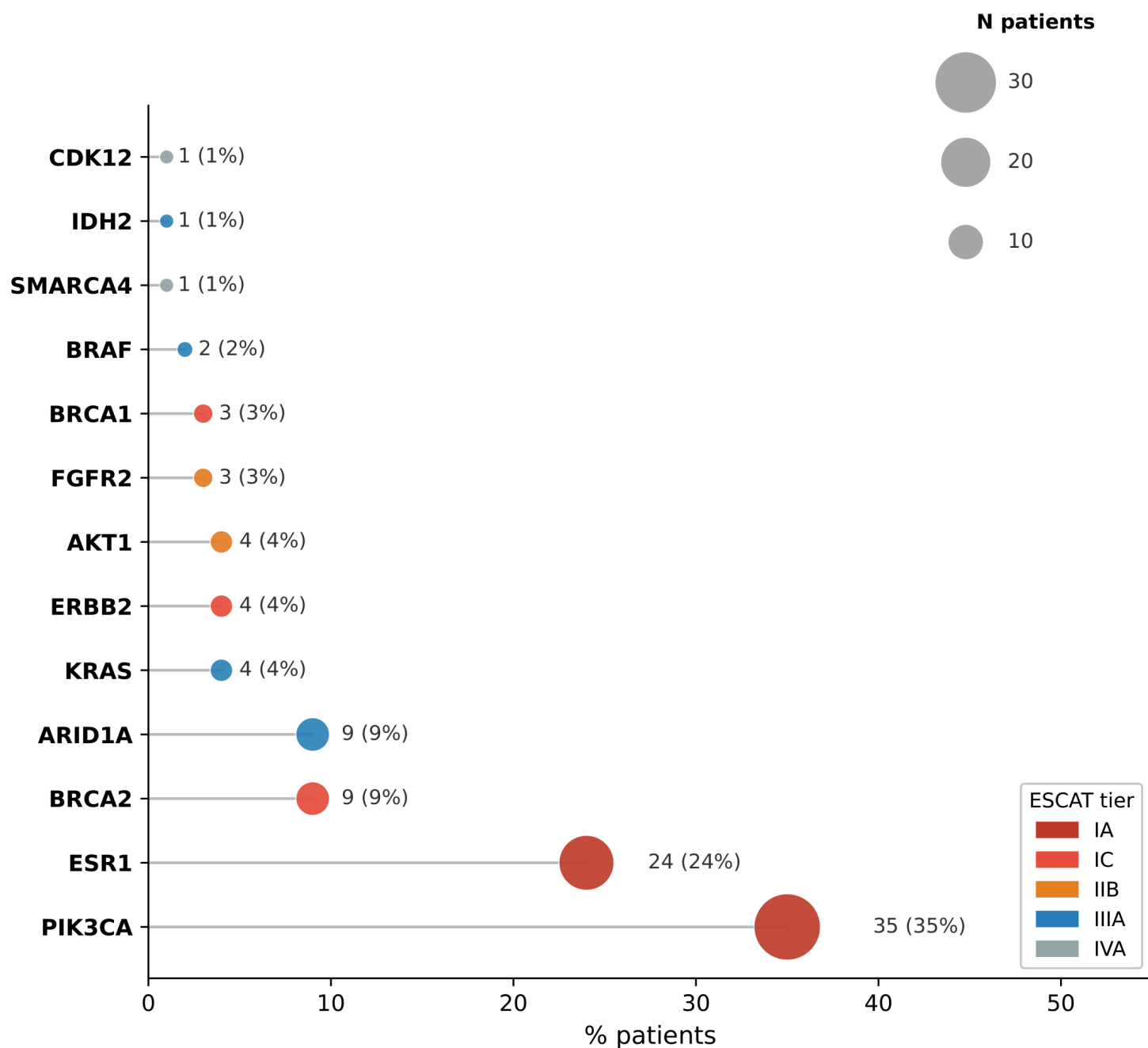


Figure 2

Actionable alterations at the first contributive liquid biopsy. Frequency of ESCAT-classified alterations detected in circulating tumor DNA at the first contributive liquid biopsy in patients with non-hypermutated tumors (n = 100). Each bubble represents a gene; the x axis and the label show the number and percentage of patients with an alteration, bubble size is proportional to the number of patients and colour indicates the ESCAT tier. ESCAT, ESMO Scale for Clinical Actionability of Molecular Targets.

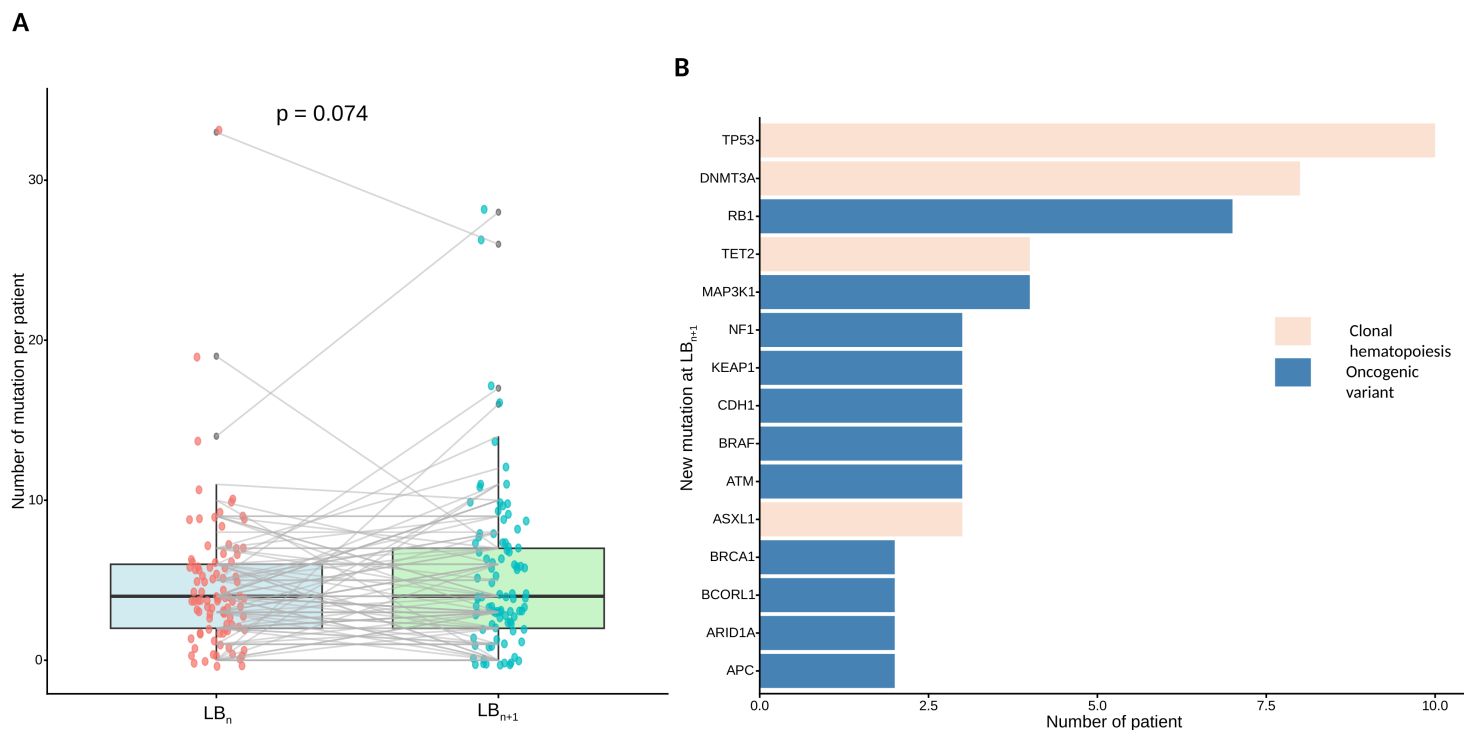


Figure 3

Mutational dynamics between consecutive contributive liquid biopsies. **a**, Number of mutations detected per patient at the first contributive liquid biopsy (LB_n) and at the next contributive liquid biopsy (LB_{n+1}). Dots represent individual patients, and gray lines connect paired samples. Box plots show the median (center line) and interquartile range (box limits); the P value was obtained with a two-sided Wilcoxon signed-rank test. **b**, Number of patients with each newly detected gene alteration at LB_{n+1} . Beige bars indicate variants associated with clonal hematopoiesis and blue bars oncogenic variants.

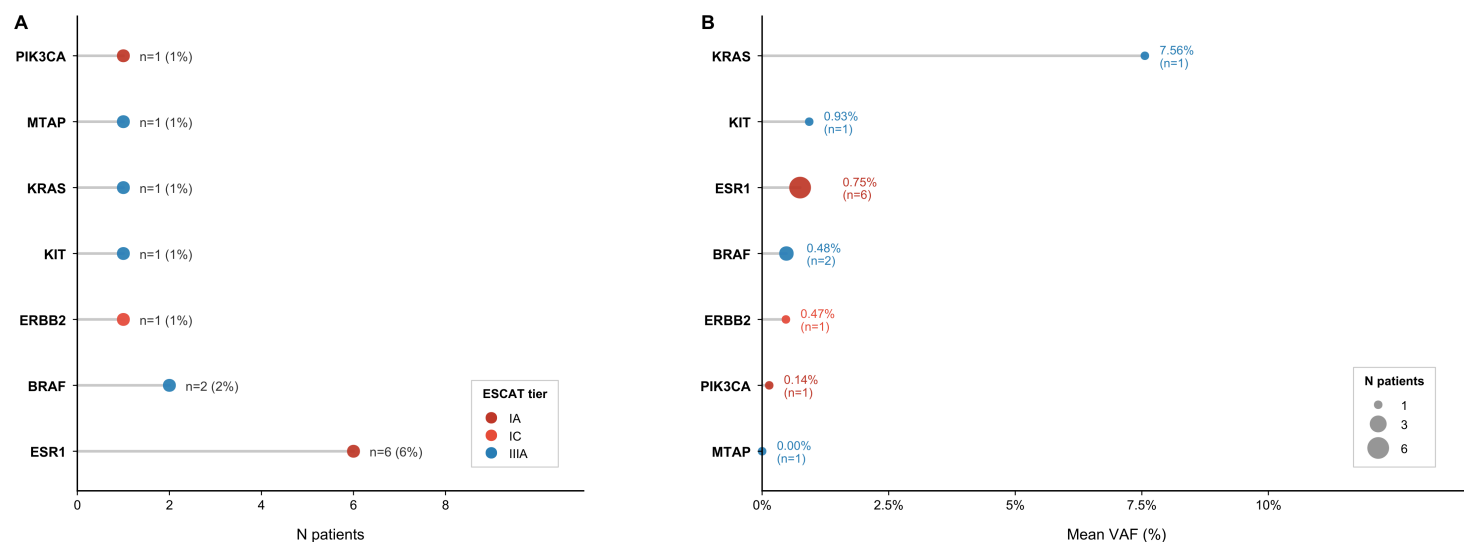


Figure 4

Concordance of ESCAT-classified alterations across serial liquid biopsies. Analysis of 75 patients with at least two contributive liquid biopsies and non-hypermuted tumors. **a**, Gene-level Matthews correlation coefficient (MCC) between consecutive contributive liquid biopsies, calculated from the presence or absence of each alteration at both timepoints. Each bubble represents a gene; bubble size is proportional to the number of patients with the alteration at the first liquid biopsy (LB1) and colour indicates the ESCAT tier. Vertical dashed lines indicate the thresholds for moderate (0.5) and high (0.8) concordance. **b**, Heat map of MCC values in all patients and in the HR+/HER2- and triple-negative breast cancer (TNBC) subgroups, grouped by ESCAT tier; empty cells indicate genes not altered in the subgroup. ESCAT, ESMO Scale for Clinical Actionability of Molecular Targets; HER2-, human epidermal growth factor receptor 2-negative; HR+, hormone receptor-positive.

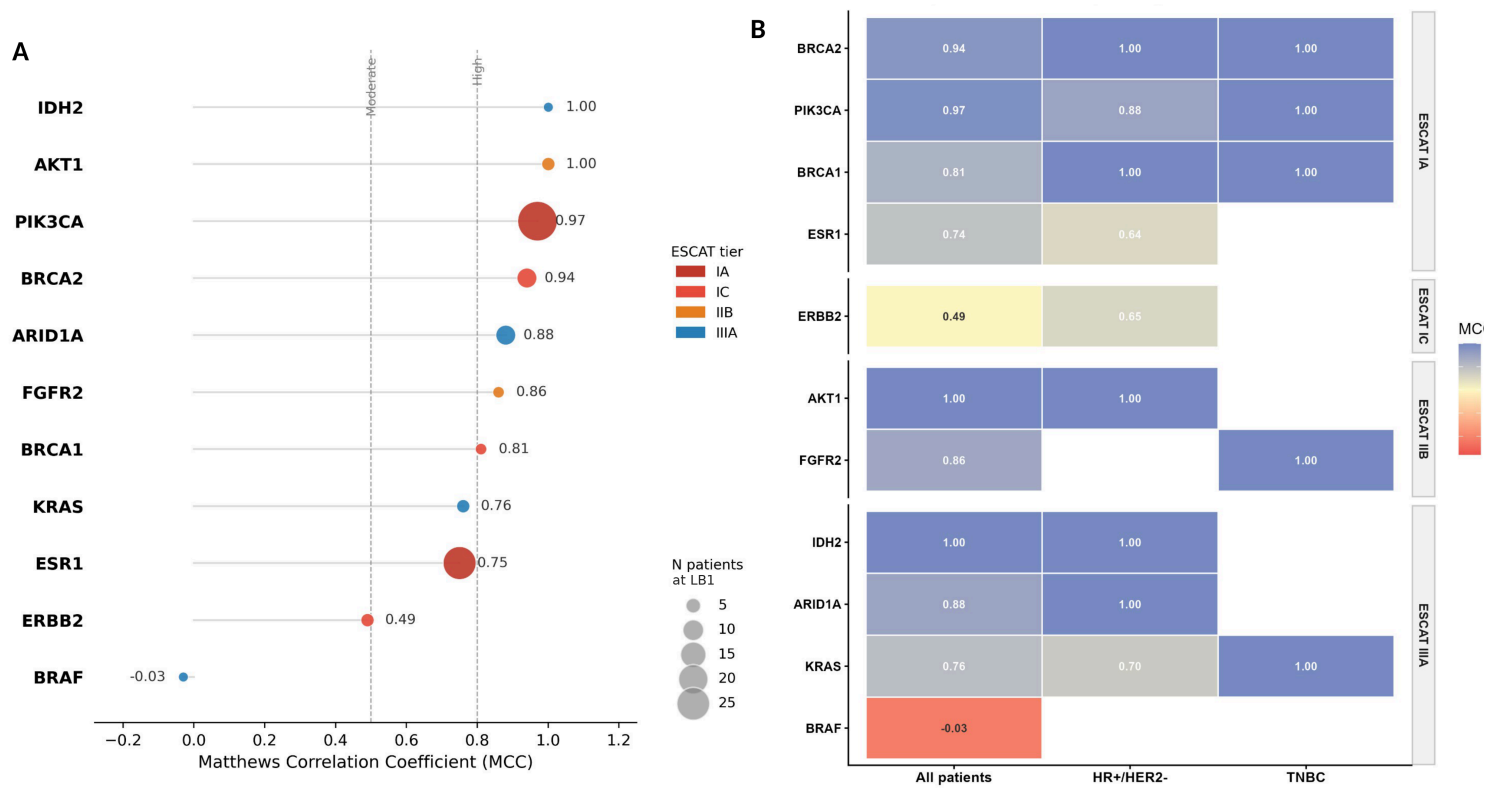


Figure 5

Newly emergent ESCAT-classified alterations. **a**, Number and percentage of patients with newly emergent ESCAT-classified alterations, by gene, at subsequent contributive liquid biopsies among patients with at least two contributive liquid biopsies (n = 96); colour indicates the ESCAT tier. **b**, Mean variant allele frequency (VAF) of newly emergent ESCAT-classified alterations per gene; bubble size is proportional to the number of patients. ESCAT, ESMO Scale for Clinical Actionability of Molecular Targets.

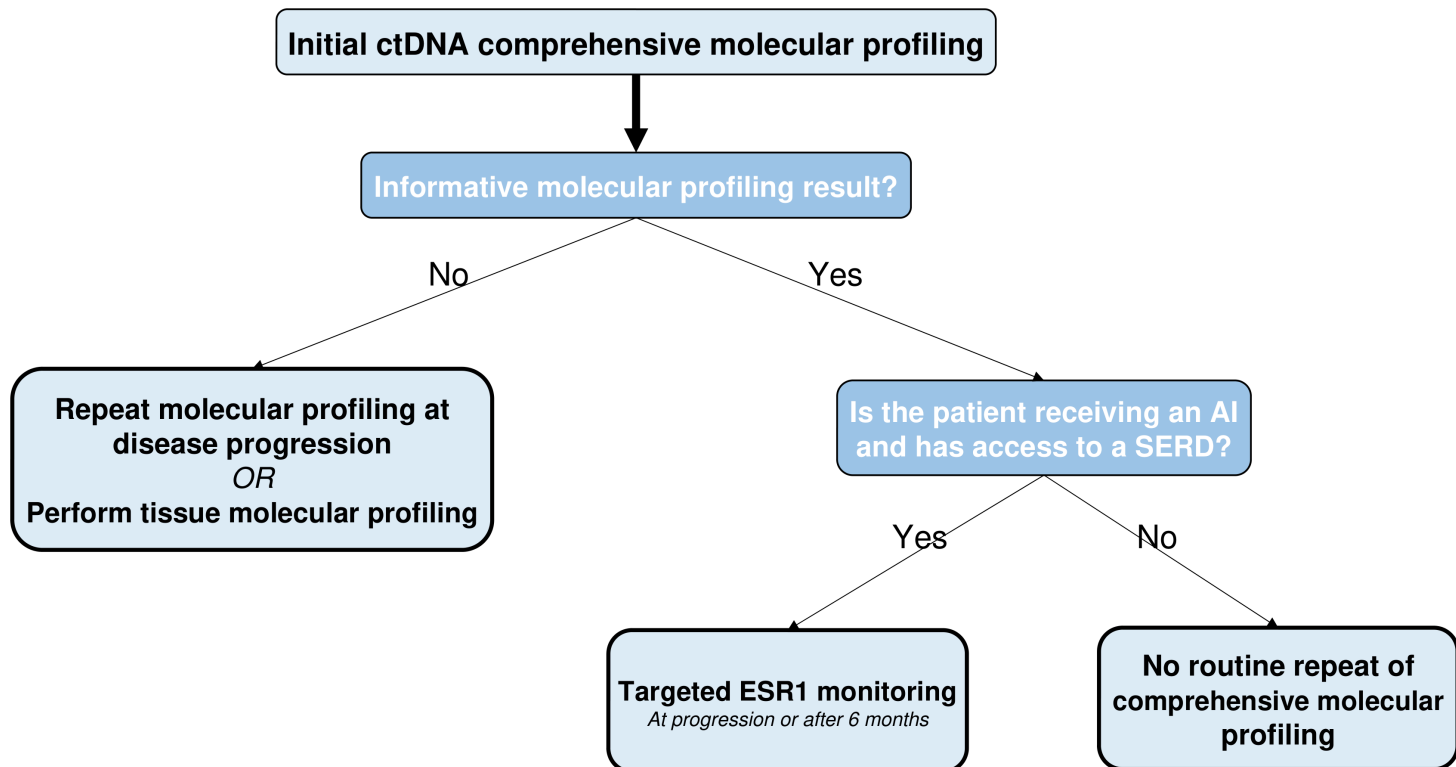


Figure 6

Proposed framework for serial ctDNA profiling in advanced breast cancer. Decision algorithm based on the contributivity of the first comprehensive circulating tumor DNA (ctDNA) assay. After a contributive assay, targeted *ESR1* monitoring, at progression or after 6 months of treatment, is proposed for patients receiving an aromatase inhibitor (AI) with access to a selective estrogen receptor degrader (SERD); otherwise, routine repeat comprehensive profiling is not recommended. After a non-contributive assay, comprehensive profiling is repeated at disease progression, or tissue molecular profiling is performed.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryData1.xlsx](#)
- [SupplementaryInformationnpjBreastCancer.pdf](#)