Circulating tumour DNA analysis to direct therapy in advanced breast cancer (plasmaMATCH): a multicentre, multicohort, phase 2a, platform trial

Nicholas C Turner, Belinda Kingston, Lucy S Kilburn, Sarah Kernaghan, Andrew M Wardley, Iain R Macpherson, Richard D Baird, Rebecca Roylance, Peter Stephens, Olga Oikonomidou, Jeremy P Braybrooke, Mark Tuthill, Jacinta Abraham, Matthew C Winter, Hannahs Bye, Michael Hubank, Heidrun Gevensleben, Ros Cutts, Claire Snowden, Daniel Rea, David Cameron, Abeer Shaaban, Katrina Randle, Sue Martin, Katie Wilkinson, Laura Moretti, Judith M Bliss*, Alistair Ring*

Summary
Background Circulating tumour DNA (ctDNA) testing might provide a current assessment of the genomic profile of advanced cancer, without the need to repeat tumour biopsy. We aimed to assess the accuracy of ctDNA testing in advanced breast cancer and the ability of ctDNA testing to select patients for mutation-directed therapy.

Methods We did an open-label, multicohort, phase 2a, platform trial of ctDNA testing in 18 UK hospitals. Participants were women (aged ≥18 years) with histologically confirmed advanced breast cancer and an Eastern Cooperative Oncology Group performance status 0–2. Patients completed at least one previous line of treatment for advanced breast cancer or relapsed within 12 months of neoadjuvant or adjuvant chemotherapy. Patients were recruited into four parallel treatment cohorts matched to mutations identified in ctDNA: cohort A comprised patients with ERα mutations (treated with intramuscular extended-dose fulvestrant 500 mg); cohort B comprised patients with HER2 mutations (treated with oral neratinib 240 mg, and if oestrogen receptor-positive with intramuscular standard-dose fulvestrant); cohort C comprised patients with AKT1 mutations and oestrogen receptor-negative cancer (treated with oral capivasertib 400 mg plus intramuscular standard-dose fulvestrant); and cohort D comprised patients with AKT1 mutations and oestrogen receptor-positive cancer or PTEN mutation (treated with oral capivasertib 480 mg). Patients had a primary endpoint of confirmed objective response rate (COR). For cohort A, 13 or more responses among 78 evaluable patients were required to infer activity and three or more among 16 were required for cohorts B, C, and D. Recruitment to all cohorts is complete and long-term follow-up is ongoing. This trial is registered with ClinicalTrials.gov, NCT03182634; the European Clinical Trials database, EudraCT2015-003735-36; and the ISRCTN registry, ISRCTN16945804.

Findings Between Dec 21, 2016, and April 26, 2019, 1051 patients registered for the study, with ctDNA results available for 1034 patients. Agreement between ctDNA digital PCR and targeted sequencing was 96–99% (n=800, kappa 0.89–0.93). Sensitivity of digital PCR ctDNA testing for mutations identified in tissue sequencing was 93% (95% CI 83–98) overall and 98% (87–100) with contemporaneous biopsies. In all cohorts, combined median follow-up was 14–4 months (IQR 7.0–23.7), Cohorts B and C met or exceeded the target number of responses, with six (8% [95% CI 3–17]) of 74 in cohort A and two (11% [1–33]) of 19 patients in cohort D having a response. The most common grade 3–4 adverse events were raised gamma-glutamyltransferase (13% [16%] of 80 patients; cohort A); diarrhoea (four [25%] of 20; cohort B); fatigue (four [22%] of 18; cohort C); and rash (five [26%] of 19; cohort D). 17 serious adverse reactions occurred in 11 patients, and there was one treatment-related death caused by grade 4 dyspnoea (in cohort C).

Interpretation ctDNA testing offers accurate, rapid genotyping that enables the selection of mutation-directed therapies for patients with breast cancer, with sufficient clinical validity for adoption into routine clinical practice. Our results demonstrate clinically relevant activity of targeted therapies against rare HER2 and AKT1 mutations, confirming these mutations could be targetable for breast cancer treatment.

Funding Cancer Research UK, AstraZeneca, and Puma Biotechnology.

Introduction Multiple tumour mutations are potentially targetable for advanced breast cancer treatment. Some of these mutations are common, such as activating PI3KCA mutations that are targetable with PI3K inhibitors, including the recently approved alpelisib. Other potentially targetable mutations, such as HER2 (also known as ERBB2) and AKT1 mutations, are rare genetic mutations that are targetable with PI3K inhibitors, including the recently approved alpelisib.
Research in context

Evidence before this study

We searched PubMed on June 11, 2020, for clinical trials published between Jan 1, 2000, and Dec 31, 2019, with the terms “circulating tumour DNA”, “cell free DNA”, “plasma DNA”, “liquid biopsy”, and “ctDNA”, with no restriction on language, and identified 212 results. Circulating tumour DNA (ctDNA) analysis in multiple retrospective trials has been shown to accurately genotype mutations found in the tumour. ctDNA analysis therefore has the potential to transform the selection of targeted therapies for patients with advanced cancer. In 2019, the TARGET trial reported a potential role for ctDNA testing in 100 patients with advanced cancers in an early-phase clinical trial setting. However, there has been uncertainty about the validity of ctDNA testing in routine practice, as there have been few large prospective studies to assess the accuracy and utility of ctDNA testing. In addition, sensitivity has not been perfect in previous retrospective studies, suggesting the potential for false negative ctDNA results, and, in routine clinical practice, reflex testing of tumour tissue is advised to confirm negative results. In 2018, the American Society of Clinical Oncology and College of American Pathologists guidelines committee on ctDNA analysis concluded that the absence of prospective trials was one of the major weaknesses in the evidence for bringing ctDNA testing to routine practice, with a need for trials that recruited patients solely on the basis of ctDNA testing without tissue testing beforehand.

Added value of this study

plasmMATCH is, to our knowledge, the first large, prospective, multicentre study assessing the feasibility and clinical utility of ctDNA analysis to direct therapy in patients with advanced breast cancer. We recruited 1051 patients for ctDNA testing, from both academic and general hospitals, and tested with two orthogonal ctDNA analysis techniques. We found high agreement between ctDNA assays, and high sensitivity for mutations identified in tissue sequencing, especially with contemporaneous biopsies. Patients with rare, potentially targetable mutations in HER2 and AKT1 in ctDNA had clinically important responses with the HER2 inhibitor neratinib and AKT inhibitor capivasertib, respectively, similar to activity seen in previous tissue sequencing-directed trials. These findings confirm that these mutations are targetable for breast cancer therapy, and demonstrate the validity and clinical utility of using ctDNA testing to screen patients for rare mutations.

Implications of all the available evidence

These findings show that ctDNA testing for mutations has sufficient accuracy for widespread adoption in clinical practice, with the assays used. The high sensitivity of ctDNA testing for tissue mutations calls into question the need for reflex tissue testing for negative ctDNA results, within the pretreated metastatic breast cancer patient population studied. This study also shows the potential of a novel liquid biopsy platform to screen for rare oncogenic mutations in breast cancer, and how this approach could transform clinical trials with efficient and rapid mutation screening.

Methods

Study design and participants

plasmMATCH is a multicohort, open-label, non-randomised, phase 2a clinical trial platform run across 18 UK hospitals (appendix p 2). Investigators at UK hospitals registered eligible patients with the Institute of Cancer Research Clinical Trials and Statistics Unit (ICR-CTSU) for ctDNA testing. Those with potentially targetable mutations identified in ctDNA testing (ESR1, HER2, AKT1, or PTEN) were offered entry into one of four parallel treatment cohorts (A–D) according to the
mutation identified, with therapies matched to mutations. A fifth cohort (E) recruiting patients with triple-negative breast cancer with no targetable mutation, designated to receive olaparib plus the ATR inhibitor AZD6738, is ongoing and will be reported separately. Eligible patients were women at least 18 years of age with histologically confirmed advanced breast cancer that was not suitable for treatment with radical or curative intent, who had measurable disease, an Eastern Cooperative Oncology Group performance status of 0–2, estimated life expectancy of more than 3 months, and were suitable for a baseline advanced disease biopsy or had an archival advanced disease biopsy available for subsequent retrospective sequencing and comparison with ctDNA.

Patients were required to have had disease progression on radiological or clinical assessment at registration (with radiological confirmation required before treatment cohort entry), and to have completed at least one previous line of treatment for advanced breast cancer, or relapsed within 12 months of neoadjuvant or adjuvant chemotherapy. Patients with HER2-positive breast cancer must have had at least two previous lines of HER2-targeted therapy in the advanced setting (or one line if no further HER2-targeted therapies were available). An approved protocol amendment implemented on Feb 19, 2018, after 515 patients had been recruited, required a maximum of two previous lines of chemotherapy, antibody–drug conjugate, or immunotherapy. Exclusion criteria for ctDNA testing included uncontrolled CNS or cardiac disease, ongoing toxicities of grade 1 or higher from previous treatments, and malignancies of other types within the past 3 years. Cohort-specific eligibility criteria are given in the protocol (appendix).

The study was co-sponsored by the Institute of Cancer Research and the Royal Marsden National Health Service (NHS) Foundation Trust, London, UK, and approved by a Research Ethics Committee (16/SC/0271). All participants gave written informed consent before registration for ctDNA testing, and again before treatment cohort entry. Safety and efficacy data were reviewed regularly by an independent data monitoring committee. Trial oversight was provided by an independent trial steering committee.

Procedures

ctDNA testing was done with two technologies. Digital droplet PCR was done at a central laboratory in the National Institute for Health Research Centre for Molecular Pathology at the Royal Marsden NHS Foundation Trust and Institute of Cancer Research prospectively in all patients, for mutations in PIK3CA, ESR1, HER2, and AKTI (appendix p 4). From July 10, 2018 (after recruitment of 680 patients), prospective testing also included error-corrected targeted sequencing with Guardant360 (Guardant Health; Redwood City, CA, USA) for a panel of 73 genes including PIK3CA, ESR1, HER2, AKTI, PTEN, and TP53, with retrospective sequencing for previously enrolled patients. For comparison with ctDNA results, tumour tissue sequencing using advanced disease tissue biopsies was done retrospectively for patients who entered a treatment cohort (appendix p 5). Testing for PIK3CA mutations was included to test the validity of the PIK3CA ctDNA testing, but was not used for entry to therapeutic cohorts as phase 3 studies of treatments for breast cancer with PIK3CA mutations were ongoing when plasmaMATCH started recruitment.1 A positive result by either ctDNA assay was sufficient for cohort entry. If more than one mutation was identified, entry to cohorts B–D took preference to cohort A. Cohort A included individuals with ESR1 mutations; they received extended-dose 500 mg fulvestrant (a selective oestrogen receptor downregulator) administered intramuscularly on days 1, 8, and 15 in cycle 1, and days 1 and 15 in cycle 2 onwards, on a 28-day cycle. Pharmacokinetic analysis samples were collected predose on cycles 2–4 and compared with a historical population model for standard-dose fulvestrant. In cohort A, as a prespecified exploratory analysis, ESR1 mutations were determined to be clonally dominant or subclonal, with a clonally dominant mutation indicating a summed ESR1 allele fraction of 50% or greater of maximum allele fraction detected in the sample by targeted sequencing to correct for variations in the purity of ctDNA in plasma DNA (appendix p 5).

Cohort B included individuals with HER2 mutations; they received 240 mg neratinib (an irreversible pan-HER tyrosine kinase inhibitor) orally once a day on a continuous schedule. In patients with oestrogen receptor-positive breast cancer, this treatment was administered together with fulvestrant 500 mg intramuscularly at standard dosing (days 1 and 15 in cycle 1 and day 1 in cycle 2 onwards on a 28-day cycle).

Cohort C included individuals with AKTI mutations and oestrogen receptor-positive breast cancer; they received 400 mg capivasertib (selective AKT inhibitor) orally twice a day for 4 days on followed by 3 days off continuously with fulvestrant 500 mg intramuscularly at standard dosing.

Cohort D included individuals with an AKT pathway activating mutation (mutations in AKTI with oestrogen receptor-negative breast cancer, or PTEN inactivating mutations or homozygous deletion [irrespective of oestrogen receptor status]; full criteria are described in the appendix p 6). Patients with mutations identified in previous tumour sequencing done outside of plasmaMATCH were also eligible for this cohort. This cohort received 480 mg capivasertib monotherapy orally, twice a day for 4 days on, followed by 3 days off, continuously.

In addition to the eligibility criteria for ctDNA testing, for cohort assignment patients with a relevant targetable mutation had to have adequate haematological, renal, and hepatic function (adequate defined as absolute neutrophil count ≥1·0×10⁹ cells per L, platelet count ≥100×10⁹ per L,
haemoglobin ≥9 g/dL, serum creatinine ≤1.5× the upper limit of normal [ULN], total bilirubin ≤1.5× ULN, alanine aminotransferase and aspartate aminotransferase ≤3× ULN [or ≤5× ULN in the presence of liver metastases]). For cohorts C and D, patients were excluded if baseline glycated haemoglobin (HbA1c) was ≥8.0% (64 mmol/mol) or fasting plasma glucose was ≥7.0 mmol/L (126 mg/dL), or they had poorly controlled diabetes. Patients were eligible for cohort entry with detection of a mutation at any allele fraction, and clonal dominance was not considered in eligibility.

Once enrolled into a cohort, treatment was given until disease progression, unacceptable toxicity, or pregnancy. Participants could also discontinue from trial treatment at any time at their own request or be discontinued at the discretion of the treating clinician. Within each cohort, dose modifications were permitted for patients experiencing toxicities related to treatment.

Patients underwent CT or MRI scan and bone scan at baseline, with CT or MRI scan repeated every 8 weeks until 32 weeks after commencing treatment, and every 12 weeks thereafter, for disease evaluation using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria. There was no independent central review of disease outcome. Laboratory assessments, adverse event recording, and vital signs were performed every 4 weeks at a minimum. Toxicity was assessed using National Cancer Institute Common Terminology Criteria for Adverse Events version 4. Coding was done with use of the Medical Dictionary for Regulatory Activities version 22.

**Outcomes**

The primary endpoint for cohorts A–D was confirmed objective response rate defined as a confirmed complete or partial response according to RECIST criteria at any point during trial treatment. Secondary endpoints included duration of response (defined as time from the first documentation of complete response or partial response until date of disease progression or last date of follow-up), clinical benefit rate (defined as complete response, partial response, or stable disease for more than 6 months during trial treatment), progression-free survival (defined as time from cohort entry to first date of either confirmed progression of disease according to RECIST criteria or death from any cause), safety and tolerability of therapies, frequency of mutations, accuracy of ctDNA testing by agreement between ctDNA mutation status and tissue mutation status and the proportion of patients entering a cohort, and pharmacokinetics (for cohorts A and B). Prespecified exploratory endpoints included confirmed response rate in clonally dominant versus subclonal mutations in cohort A.

**Statistical analysis**

All cohorts used a single-stage A’Hern design with α 5%, to have 80% power. Cohort A assumed an unacceptable response rate of 10% and a target response rate of 20% in the final design, requiring 13 or more responses from 78 evaluable patients to infer activity. This assumption was an approved amendment (on May 1, 2018) to the original two-stage design to account for the ctDNA testing detecting subclonal mutations as well as clonal mutations where the response rate was expected to be lower (appendix p 6). Cohorts B, C, and D each assumed an unacceptable response rate of 5% and a target response rate of 25%, requiring three or more responses from 16 evaluable patients. Over-recruitment into cohorts B, C, and D was allowed while ctDNA testing remained active. We estimated that 1000 patients would be required to enter ctDNA testing to recruit sufficient patients for cohorts B and C. This number gave 85% probability of identifying 25 patients for a mutation with prevalence of 3% for each of cohorts B, C, and D individually, allowing for 36% attrition between ctDNA screening and cohort entry.

Objective response rate, duration of response, and clinical benefit rate were measured in an evaluable population defined as those patients with measurable disease per RECIST at baseline and at least one on-treatment assessment; patients who stopped treatment because of intolerable toxicity or death without having a scan after baseline were evaluable and recorded as non-responders. Proportions and two-sided 95% CIs for estimation purposes were reported for each cohort and in prespecified subgroup analyses (by clonally dominant versus subclonal mutations in cohort A, compared using a Fisher’s exact test, by hormone receptor status in cohort B, and by AKT1 or PTEN mutation in cohort D). Analysis of clonality of HER2 mutations in cohort B and of AKT1 in cohort C was a post-hoc analysis.

In post-hoc exploratory analyses, response rates for each cohort were reported by PIK3CA and TP53 mutation status and, for cohort A, tissue mutation status. For inference purposes, thus corresponding to the design characteristics underpinning the trial’s hypothesis testing (ie, alpha 5%, one-sided), proportions and two-sided 90% CIs are reported.

Progression-free survival was measured in the intention-to-treat population. Kaplan-Meier curves were plotted and median progression-free survival is reported with 95% CIs for each cohort. Patients who were alive and progression free were censored at date of last follow-up; patients who had non-RECIST confirmed progression (eg, clinically judged progression or radiologically confirmed but lesions not measured according to RECIST) were censored at the date progression was reported. The safety population included all patients who had at least one dose of treatment, regardless of their eligibility, and treatment-emergent adverse events where more than 10% of patients reported any grade of the adverse event or any patients reported the adverse event at grade 3 or higher were presented. In addition, pharmacokinetics were reported as a percentage
change from an approved historical population pharmacokinetic model for standard-dosing 500 mg fulvestrant, in cohort A only. Pharmacokinetic data for cohort B will be reported elsewhere. Safety was assessed on the basis of the incidence of adverse events.

Agreement between digital PCR and targeted sequencing was assessed with a kappa score and 95% CIs. Comparison of ctDNA with retrospective tissue biopsy sequencing was reported with sensitivity, specificity, and 95% CIs.

Analyses used a database snapshot taken on Nov 6, 2019. Where reported, p values of less than 0·05 were deemed significant. All analyses were conducted using Stata (version 15.1). This study is registered with ClinicalTrials.gov, NCT03182634; the European Clinical Trials database, EudraCT2015-003735-36; and the ISRCTN registry, ISRCTN167945804.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. AstraZeneca and Puma Biotechnology reviewed the final version of the report, but had no role in the decision to submit the manuscript for publication. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results
Between Dec 21, 2016, and April 26, 2019, 1051 patients were registered into the study (1044 via ctDNA screening, seven via previous tumour sequencing; figure 1; appendix p 17) with ctDNA results available for 1034 patients (99·0%). Digital PCR results were available for 800 patients (76·6%; 364 prospective and 436 retrospective). The median time from blood draw to ctDNA results was 13 days (IQR 11–15) for digital PCR and 10 days (8–11) for sequencing. Patients had a median of one (IQR 0–2) previous lines of chemotherapy, and a median of two (1–3) previous lines of systemic therapy (table).

A somatic mutation was detected in 743 (93%) of 800 patients with ctDNA targeted sequencing results (appendix p 18). ESR1 mutations were found almost exclusively in hormone receptor-positive breast cancer; were found at lower average allele fractions than other mutations, and were frequently polyclonal (appendix pp 17–18). HER2 mutations were found least frequently in triple-negative breast cancer, while AKT1 mutations were found at a similar frequency in hormone receptor-negative HER2-negative breast cancer and triple-negative breast cancer (appendix p 18).

Gene-level agreement for mutation identification between ctDNA digital PCR and targeted sequencing (n=800) was 96–99% (kappa 0·89–0·93; figure 1). Individual mutation agreements were also high (appendix p 19). An advanced tissue biopsy was sequenced retrospectively for 77 patients who entered a cohort. Sensitivity (or percent-positive agreement reflecting the absence of gold standard) for digital PCR was 93% (95% CI 83–98) overall and 98% (87–100) in patients with contemporaneous biopsies (appendix pp 17, 20). By contrast, digital PCR sensitivity in patients with time-discordant biopsies was 85% (95% CI 66–96; appendix p 20). Sensitivity for targeted sequencing was 95% (95% CI 87–99) overall and 100% (92–100) in patients with contemporaneous biopsies (appendix p 21). Sensitivity for time-discordant biopsies is show in the appendix (p 20).

Specificity for both digital PCR and sequencing (or percent-negative agreement reflecting the absence of gold standard) was high for AKT1, HER2, and PIK3CA, varying by gene (appendix p 17). ESR1 mutations had lower percent-negative agreement.

Mutations were identified in 533 (51·1%) of 1044 patients registered for ctDNA testing and 357 (34·5%) of 1034 with results had targetable mutations eligible for cohort entry, of whom 136 entered one of the five available cohorts. In patients who entered cohorts A–D, combined median follow-up was 14·4 months (IQR 7·0–23·7). The most common reason for not entering a cohort was that the patient was ineligible based on the specific eligibility criteria for the relevant cohort, or in the case of cohort A (ESR1 mutation), 64 patients did not enter because of cohort A being suspended (while the protocol amendment to change the design was ongoing) or closed (figure 1; appendix p 7).

84 (38%) of 222 patients with an ESR1 mutation in ctDNA identified while cohort A was open to recruitment were enrolled in cohort A (figure 1, table). The most common ESR1 mutations detected in plasma were Asp538Gly (45 [54%] of 84), Tyr537Ser (31 [37%]), and Glu380Gln (29 [35%]). 74 patients were evaluable for response: four patients did not start treatment and six did not have on-treatment RECIST-assessable imaging. Six (8% [95% CI 3–17]) of 74 patients had a confirmed partial response with a median duration of response of 7·0 months (IQR 3·7–8·3) and four patients continuing on treatment at data cutoff (figure 2). The clinical benefit rate was 12 (16% [95% CI 9–27]) of 74 patients. 69 (82%) of 84 patients had a RECIST-confirmed progression event or death. Median progression-free survival was 2·2 months (95% CI 1·8–3·6; appendix p 22). In a pre-planned exploratory analysis, five (12% [95% CI 4–26]) of 41 patients with clonally dominant ESR1 mutations, and none (0% [0–13]) of 27 patients with subclonal mutations had a confirmed response (p=0·15; 27 [40%] of 68 ESR1 mutations were subclonal); six patients had unknown clonality. The most common grade 3 or grade 4 adverse event was increased gamma-glutamyltransferase (13 [16%] of 80 patients; appendix p 9). One patient had a serious adverse reaction: grade 3 superior sagital sinus thrombosis. There were no treatment-related deaths and 41 (49%) all-cause deaths reported (38 breast cancer and three unknown cause). The main reason for treatment...
1051 met inclusion criteria and registered for plasmaMATCH

1044 registered for ctDNA testing

10 results not available (test failed or not done)

1034 ctDNA testing results available
1025 digital PCR
364 targeted sequencing

501 with no mutation or amplification identified

533 with mutation or amplification identified

376 not eligible for cohort
148 PIK3CA mutation
20 HER2 amplification
8 PIK3CA mutation and HER2 amplification

357 with targetable mutation(s) identified

221 did not enter a cohort
67 ineligible
22 patient choice
40 clinician decision
64 cohort A suspended or closed
6 other cohort closed (deadline passed for cohort entry)
8 died before cohort entry
14 unknown

136 entered a cohort via ctDNA testing

84 entered cohort A (ESR1 mutation)
80 initiated treatment
76 were evaluable for response

21 entered cohort B (HER2 mutation)
20 initiated treatment
20 were evaluable for response

18 entered cohort C (AKT1 mutation and oestrogen receptor-positive breast cancer)
18 initiated treatment
18 were evaluable for response

19 entered cohort D (AKT basket, AKT1 mutation with oestrogen receptor-negative breast cancer, or PTEN mutation)
19 initiated treatment
19 were evaluable for response

1 entered cohort E, to be reported at a later date (triple-negative breast cancer with no mutation)

Figure 1: Trial profile
Further detail on accuracy of ctDNA testing is provided in the appendix (p 17).
ctDNA = circulating tumour DNA. *436 additional samples were analysed by targeted sequencing retrospectively, these were not used for determining cohort suitability, agreement between digital PCR and targeted sequencing (n=800) was as follows:
AKT1 kappa 0.93 (95% CI 0.87–0.99),
HER2 kappa 0.89 (0.79–0.98),
ESR1 kappa 0.90 (0.86–0.93), and
PIK3CA kappa 0.92 (0.89–0.95).
discontinuation was disease progression (72 [95%] of 76 patients; appendix p 8). Two patients had reductions of fulvestrant dosing frequency. 29 patients did not have a day 15 injection given on at least one occasion. Ten delays to treatment were reported in nine patients. The median relative dose intensity in patients starting treatment was 100% (IQR 97–100, range 33–104). Pharmacokinetic analysis was consistent with elevated fulvestrant exposure compared with standard-dosing 500 mg fulvestrant in a historical population pharmacokinetic model (appendix p 10). Fulvestrant activity was similar in patients with and without ESR1 mutations in tissue sequencing (appendix p 29).

21 (58%) of 36 patients with an HER2 mutation in cDNA were enrolled in cohort B (figure 1, table). The most common HER2 mutations detected in plasma were Leu755Ser (ten [48%] of 21 patients), Val777Leu (four [19%]), and Ser310Phe (three [14%]). 20 patients were evaluable for response, as one patient did not start treatment. Five (25% [95% CI 9–49]) of 20 patients had a confirmed response (one complete and four partial), and an additional three patients had unconfirmed partial responses (figure 3). Four (25% [95% CI 7–52]) of the protocol-specified first 16 evaluable patients had a response. One patient had a complete response, ongoing at 29 months duration. Median duration of response was 5.7 months (IQR 3.7–9.7) with three patients continuing on treatment at data cutoff. The clinical benefit rate was nine (45% [95% CI 23–68]) of 20 patients. 16 (76%) of 21 patients had a RECIST-confirmed progression event or...

| Age group at registration (years) | All registered patients (n=1051) | Patients with ESR1 mutation (n=222) | Patients with HER2 mutation (n=36) | Patients with oestrogen receptor-positive breast cancer and AKT1 mutation (n=30) | Patients with oestrogen receptor-negative breast cancer and AKT1 mutation or PTEN mutation (n=37) |
|----------------------------------|----------------------------------|-----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| <50                              | 285 (27.1%)                      | 18 (21.4%)                        | 2 (9.5%)                         | 3 (16.7%)                        | 2 (16.7%)                        |
| 50-59                            | 348 (33.1%)                      | 36 (42.9%)                        | 9 (42.9%)                        | 3 (20.0%)                        | 7 (25.0%)                        |
| 60-69                            | 265 (25.2%)                      | 20 (23.8%)                        | 6 (28.6%)                        | 8 (53.3%)                        | 6 (33.3%)                        |
| ≥70                              | 153 (14.6%)                      | 10 (11.9%)                        | 4 (19.0%)                        | 2 (13.3%)                        | 2 (11.1%)                        |
| Metastatic disease present at diagnosis | 146 (13.9%)                  | 18 (21.4%)                        | 2 (9.5%)                         | 5 (27.8%)                        | 2 (16.7%)                        |
| Histological type at tumour diagnosis |                                   |                                   |                                  |                                  |                                  |
| Ductal                           | 777 (73.9%)                      | 63 (75.0%)                        | 9 (42.9%)                        | 13 (72.2%)                       | 9 (75.0%)                        |
| Lobular                          | 98 (9.3%)                        | 10 (12.8%)                        | 8 (38.1%)                        | 3 (20.0%)                        | 3 (16.7%)                        |
| Mixed ductal and lobular         | 38 (3.6%)                        | 5 (6.0%)                          | 1 (4.8%)                         | 2 (13.3%)                        | 1 (5.6%)                         |
| Mixed ductal and mucinous        | 3 (0.3%)                         | 1 (1.2%)                          | 0                                | 0                                | 0                                |
| Other invasive                   | 13 (1.2%)                        | 2 (1.4%)                          | 0                                | 0                                | 0                                |
| Ductal carcinoma in situ         | 2* (0.2%)                        | 1 (1.2%)                          | 0                                | 0                                | 0                                |
| Not known or missing             | 120 (11.4%)                      | 5 (6.0%)                          | 3 (14.3%)                        | 1 (5.6%)                         | 1 (5.3%)                         |
| Tumour grade                     |                                   |                                   |                                  |                                  |                                  |
| 1                                | 48 (4.6%)                        | 7 (8.3%)                          | 2 (9.5%)                         | 1 (5.6%)                         | 1 (5.3%)                         |
| 2                                | 402 (38.2%)                      | 37 (44.0%)                        | 11 (52.4%)                       | 6 (40.0%)                        | 11 (61.1%)                       |
| 3                                | 445 (42.3%)                      | 28 (33.3%)                        | 4 (19.0%)                        | 5 (33.3%)                        | 5 (27.8%)                        |
| Not known or missing             | 156 (14.8%)                      | 12 (14.3%)                        | 4 (19.0%)                        | 2 (10.0%)                        | 0                                |
| Molecular subtype†               |                                   |                                   |                                  |                                  |                                  |
| HR positive, HER2 negative       | 676 (64.3%)                      | 80 (95.2%)                        | 17 (81.0%)                       | 9 (60.0%)                        | 16 (88.9%)                       |
| HR positive, HER2 positive       | 65 (6.2%)                        | 3 (3.6%)                          | 1 (4.8%)                         | 1 (6.7%)                         | 1 (5.6%)                         |
| HR negative, HER2 positive       | 36 (3.4%)                        | 0                                 | 2 (9.5%)                         | 2 (12.3%)                        | 0                                |
| Triple-negative breast cancer    | 179 (17.0%)                      | 0                                 | 1 (4.8%)                         | 1 (6.7%)                         | 3 (15.0%)                        |
| HR positive, HER2 unknown        | 39 (3.7%)                        | 7 (5.1%)                          | 0                                | 1 (6.7%)                         | 3 (15.0%)                        |
| Other†                           | 14 (1.3%)                        | 1 (0.7%)                          | 0                                | 0                                | 0                                |
| Not known or missing             | 42 (4.0%)                        | 0                                 | 0                                | 0                                | 0                                |
| Disease sites at diagnosis       |                                   |                                   |                                  |                                  |                                  |
| Visceral                         | 824 (78.4%)                      | 78 (92.9%)                        | 18 (85.7%)                       | 12 (80.0%)                       | 17 (94.4%)                       |
| Soft tissue or nodal             | 668 (63.6%)                      | 56 (66.7%)                        | 12 (57.1%)                       | 11 (73.3%)                       | 11 (61.1%)                       |
| Bone                             | 638 (60.7%)                      | 76 (90.5%)                        | 16 (76.2%)                       | 9 (60.0%)                        | 18 (100.0%)                      |

(Table continues on next page)
| Treatment received for locally advanced or metastatic disease before study registration | All registered patients (n=1051) | Patients with ESR1 mutation (n=222) | Patients with HER2 mutation (n=36) | Patients with oestrogen receptor-positive breast cancer and AKT1 mutation (n=30) | Patients with oestrogen receptor-negative breast cancer and AKT1 mutation or PTEN mutation (n=37) |
|---|---|---|---|---|---|
| | Entered cohort A (n=84) | Did not enter cohort A (n=11) | Entered cohort B (n=21) | Did not enter cohort B (n=15) | Entered cohort C (n=18) | Did not enter cohort C (n=12) | Entered cohort D (n=19) | Did not enter cohort D (n=18) |
| Chemotherapy | 728 (69.3%) | 55 (65.5%) | 88 (68.3%) | 18 (85.7%) | 10 (66.7%) | 15 (83.3%) | 8 (66.7%) | 12 (63.2%) | 9 (50.0%) |
| 1 line | 345 (32.8%) | 26 (31.0%) | 38 (27.5%) | 7 (33.3%) | 4 (26.7%) | 8 (44.4%) | 5 (41.7%) | 7 (36.8%) | 4 (22.2%) |
| 2 lines | 201 (19.1%) | 13 (15.5%) | 27 (19.6%) | 9 (42.9%) | 3 (20.0%) | 3 (16.7%) | 3 (25.0%) | 4 (21.1%) | 3 (16.7%) |
| >2 lines$ | 181 (17.2%) | 16 (19.0%) | 22 (15.9%) | 2 (9.5%) | 3 (20.0%) | 4 (22.2%) | 0 | 1 (5.3%) | 2 (11.1%) |
| Unknown | 1 (0.1%) | 0 | 0 (0.7%) | 0 | 0 | 0 | 0 | 0 | 0 |
| Endocrine therapy¶ | 685 (65.2%) | 79 (94.0%) | 127 (92.0%) | 14 (66.7%) | 11 (73.3%) | 18 (100%) | 12 (100.0%) | 11 (84.6%) | 7 (63.6%) |
| 1 line | 323 (30.7%) | 34 (40.5%) | 45 (32.6%) | 3 (14.3%) | 3 (20.0%) | 9 (59.0%) | 8 (66.7%) | 8 (65.5%) | 4 (32.7%) |
| 2 lines | 230 (21.9%) | 36 (42.9%) | 49 (35.5%) | 6 (28.6%) | 5 (33.3%) | 6 (33.3%) | 1 (8.3%) | 3 (23.1%) | 4 (34.6%) |
| 3 lines | 117 (11.1%) | 8 (9.5%) | 30 (21.7%) | 5 (23.8%) | 2 (13.3%) | 3 (16.7%) | 3 (25.0%) | 0 | 0 |
| >3 lines | 15 (1.4%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total lines of systemic therapy | 0 | 93 (8.9%) | 2 (2.4%) | 6 (4.3%) | 0 | 1 (6.7%) | 0 | 0 | 3 (15.8%) | 6 (33.3%) |
| 1 | 275 (26.2%) | 15 (17.9%) | 25 (18.1%) | 3 (14.3%) | 3 (20.0%) | 1 (5.6%) | 3 (25.0%) | 5 (26.3%) | 3 (16.7%) |
| 2 | 243 (23.1%) | 23 (27.4%) | 21 (15.7%) | 4 (19.0%) | 3 (20.0%) | 6 (33.3%) | 2 (16.7%) | 5 (26.3%) | 5 (27.8%) |
| 3 | 178 (16.9%) | 16 (19.0%) | 28 (20.3%) | 8 (38.1%) | 4 (26.7%) | 5 (27.8%) | 5 (41.7%) | 4 (21.1%) | 2 (11.1%) |
| 4 | 109 (10.4%) | 12 (14.3%) | 18 (13.0%) | 4 (19.0%) | 2 (13.3%) | 3 (16.7%) | 2 (16.7%) | 2 (10.5%) | 0 |
| 5 | 88 (8.4%) | 11 (13.1%) | 19 (13.8%) | 2 (9.5%) | 0 | 0 | 0 | 0 | 2 (11.1%) |
| >5 | 65 (6.2%) | 5 (6.0%) | 12 (8.7%) | 0 | 2 (13.3%) | 3 (16.7%) | 0 | 0 | 0 |
| Other systemic therapy¶ | 421 (40.1%) | 41 (48.8%) | 59 (42.8%) | 11 (54.2%) | 8 (53.3%) | 9 (50.0%) | 4 (33.3%) | 11 (57.9%) | 7 (38.9%) |
| Anti-HER2 therapy | 89 (8.5%) | 3 (3.6%) | 1 (0.7%) | 3 (14.3%) | 3 (20.0%) | 1 (5.6%) | 0 | 0 | 0 |
| mTOR inhibitor (everolimus or vistusertib) | 116 (11.0%) | 18 (21.4%) | 31 (22.5%) | 5 (23.8%) | 2 (13.3%) | 3 (16.7%) | 1 (8.3%) | 3 (15.8%) | 2 (11.1%) |
| CDK4/6 inhibitor (palbociclib, ribociclib, or abemaciclib) | 89 (8.5%) | 8 (9.5%) | 14 (10.1%) | 1 (4.8%) | 1 (6.7%) | 6 (33.3%) | 0 | 4 (21.1%) | 1 (5.6%) |
| Immunotherapy (atezolizumab or pembrolizumab) | 20 (1.9%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other | 45 (4.3%) | 1** (1.2%) | 6 (4.3%) | 1** (4.8%) | 2 (13.3%) | 0 | 3 (25.0%) | 2** (10.5%) | 1 (5.6%) |

Data are n (%). HR=hornone receptor (oestrogen or progesterone receptor). $Two patients originally had ductal carcinoma in situ only as their primary diagnosis, but relapsed with invasive advanced cancer. $Determined at local hospitals from recurrence biopsy (or primary biopsy if recurrence biopsy unavailable). $Other molecular subtypes were: four HR negative, HER2 unknown, nine HR unknown, HER2 negative, one HR unknown, HER2 positive. ¶Study was amended after 515 patients had been recruited to require a maximum of two previous lines of chemotherapy. ¶¶Or patients with oestrogen receptor-negative breast cancer and AKT1 mutation or PTEN mutation the denominator is patients with HR-positive disease only (for those who entered cohort D n=13; for those that did not enter cohort D n=11). ||Patients may be included in more than one type of systemic therapy, but patients are only included once in each category (eg, if a patient had trastuzumab and pertuzumab they are counted once in the anti-HER2 therapy category). ** Tisobuvir. |||Capivasertib. |||L��tucib (n=1) and olaparib (n=1). Table: Baseline characteristics

Death. Median progression-free survival was 5-4 months (95% CI 3.4–9.1; appendix p 23). In the subgroup of patients with hormone receptor-positive HER2-negative breast cancer treated with neratinib and fulvestrant, four (24% [95% CI 7–50]) of 17 patients had a confirmed response (figure 3). The single triple-negative patient with HER2 mutation did not respond. In a post-hoc analysis, three (16%) of 19 HER2 mutations were subclonal (appendix p 24). The most common grade 3 or grade 4 adverse events were diarrhea (four [20%] of 20 patients) and hypertension (three [15%]; appendix p 11). Four serious adverse reactions were reported in three patients (appendix pp 11–12). There were no treatment-related deaths and 13 (62%) all-cause deaths reported (12 breast cancer and one unknown cause). The main reason for treatment discontinuation was disease progression (16 [94%] of 17 patients; appendix p 8). All 17 patients with oestrogen receptor-positive breast cancer who started treatment received all doses of fulvestrant. Neratinib dose starting treatment was 92% (IQR 84–99; range 59–100) for neratinib and 100% (100–100; 96–102) for fulvestrant.
18 (60%) of 30 patients with an AKT1 mutation in ctDNA and oestrogen receptor-positive cancer were enrolled in cohort C (figure 1; table). The most common mutation detected was Glu171Lys (17 [94%] of 18 patients), and Leu52Arg was detected in one patient (6%). All 18 patients were evaluable; four (22% [95% CI 6–48]) patients had a confirmed partial response, and an additional four patients had unconfirmed partial responses (figure 4A). Three (19% [4–46]) of the protocol-specified first 16 evaluable patients had a response. Median duration of response was 7.5 months (IQR 4–10) with four patients continuing on treatment at data cutoff. The clinical benefit rate was seven (39% [95% CI 17–64]) of 18 patients. 12 (67%) of 18 patients had a RECIST-confirmed progression event or death. Median progression-free survival was 10.2 months (95% CI 3.3–24.0; appendix p 15). In a post-hoc analysis, four (23%) of 17 AKT1 mutations (clonality was assessable in 17 patients) were subclonal (appendix p 24). The most common grade 3 or 4 adverse events were fatigue in 17 patients (95% CI 3.2–18.2; appendix p 25). In a post-hoc analysis, four (22% [95% CI 6–48]) patients had a clinical benefit. Two (11% [95% CI 1–33]) of 19 patients had a confirmed partial response, and there were two further unconfirmed responses in these patients. None of the patients with AKT1 genomic alterations responded. Two (13% [95% CI 2–38]) of the protocol-specified first 16 patients responded. Median duration of response was 3.9 months (IQR 3–7–4.2) with one patient continuing on treatment at data cutoff. Two (11% [95% CI 1–33]) of 19 patients had a clinical benefit. 13 (68%) of 19 patients had a RECIST-confirmed progression event or death. Median progression-free survival was 3.4 months (95% CI 1.8–5.5; appendix p 26). The most common grade 3 or grade 4 adverse events were rash (five [26%] of 19 patients), hypertension (two [11%]), aminotransferase increase (two [11%]), gamma-glutamyltransferase increase (two [11%]), and vomiting (two [11%]; appendix p 15). Four serious adverse reactions were reported in three patients (appendix p 15). There were no treatment-related deaths and ten (53%) breast cancer deaths reported. The main reason for treatment discontinuation was disease progression (11 [85%] of 14 patients; appendix p 8). All 18 patients received all doses of fulvestrant. Capivasertib dose was reduced to 320 mg in seven (39%) of 18 patients, and three of these patients had a further reduction to 240 mg. The median relative dose intensity was 88% (IQR 70–99, range 25–101) for capivasertib and 99% (IQR 97–100, range 94–102) for fulvestrant. 12 (67%) of 19 patients had a RECIST-confirmed progression event or death. Median progression-free survival was 3.4 months (95% CI 1.8–5.5; appendix p 26). In a post-hoc analysis, four (23%) of 17 AKT1 mutations (clonality was assessable in 17 patients) were subclonal (appendix p 24). The most common grade 3 or 4 adverse events were fatigue in 17 patients (95% CI 3.2–18.2; appendix p 25). In a post-hoc analysis, four (23%) of 17 AKT1 mutations (clonality was assessable in 17 patients) were subclonal (appendix p 24). The most common grade 3 or 4 adverse events were fatigue in 17 patients (95% CI 3.2–18.2; appendix p 25).
targetable mutations and these patients were recruited into cohorts that were given targeted therapies (matched to mutations) without confirmatory tumour testing, with activity comparable to previous studies involving tumour tissue testing.\(^4,5\) We enrolled more than 1000 patients across the UK in less than 3 years, and the dynamic trial platform design allowed for the simultaneous evaluation of multiple targeted treatment options.

The availability and accuracy of ctDNA testing shown in this study compares favourably with tissue-based mutation testing. Nearly all patients (99%) received a result from ctDNA testing, contrasting with previous tumour sequencing studies where results were typically received in only 70–90% of patients.\(^6,7\) In addition, previous tumour sequencing studies generally only included patients with disease that could be biopsied, which is not a constraint for ctDNA testing. Results were received relatively quickly after blood draw, compared with results for tissue-based testing, and this led to a high conversion rate of patients with ctDNA mutations into the corresponding treatment cohort. The accuracy of ctDNA testing was also similar to that achieved with tissue sequencing.\(^7\) Discordance between ctDNA results was still observed for patients at low allele frequency mutations, suggesting further potential for assay development. ESR1 mutations had lower percent-negative agreement, probably reflecting the subclonality of acquired ESR1 mutations, with ctDNA detecting mutations present in metastatic sites other than the one biopsied. Nevertheless, the degree of sensitivity observed in this study suggests that, within the patient population of advanced disease patients recruited, ctDNA testing could replace tissue-based mutation analysis. However, we note that tissue biopsy will remain important for immunohistochemistry, and for copy number-based assessment. Digital PCR offered similar accuracy to sequencing, with substantial cost efficiency, although this comparison was limited to the specific mutations analysed. The academic clinical laboratory doing the digital PCR assay achieved the trial target turnaround time of results within 14 days. A shorter turnaround time could easily be achieved if required in clinical practice, resulting in a cost-efficient method of ctDNA analysis.\(^3\) 533 (51.1%) of 1044 patients who underwent ctDNA testing had a potentially targetable mutation (PIK3CA, ESR1, HER2, AKT1, or PTEN), indicating a potential value for ctDNA testing.

In post-hoc analyses, the response rates in cohorts A–D did not vary by PIK3CA or TP53 co-mutational status (appendix pp 27–28).

**Discussion**

In this large, prospective trial of ctDNA testing in advanced breast cancer, we found that ctDNA testing was highly accurate, with high agreement between different ctDNA testing techniques, and high sensitivity for mutations identified in advanced breast cancer tissue biopsies. ctDNA testing identified patients with rare

![Figure 4: Capivasertib in AKT1-mutant and PTEN-mutant breast cancer (cohorts C and D)](image)

(A) Waterfall plot of maximum change in tumour size in individual patients with HR-positive cancer and AKT2 mutations in ctDNA, treated with capivasertib plus fulvestrant (cohort C). (B) Waterfall plot of maximum change in tumour size in individual patients with AKT1 mutations and HR-negative breast cancer, or with activating PTEN mutations, treated with capivasertib (cohort D). ctDNA=circulating tumour DNA. HR=hormone receptor. PTEN truncating=PTEN truncating nonsense or frameshift mutation. PTEN deletion=PTEN homozygous deletion.

In this large, prospective trial of ctDNA testing in advanced breast cancer, we found that ctDNA testing was highly accurate, with high agreement between different ctDNA testing techniques, and high sensitivity for mutations identified in advanced breast cancer tissue biopsies. ctDNA testing identified patients with rare
activity in patients with ctDNA-identified AKT1 mutations, both in hormone receptor-positive cancer with fulvestrant and in hormone receptor-negative cancer as a single agent, again confirming the results of a previous phase 1 study.7 These results confirm the high activity of these drugs against HER2 and AKT1 mutations, and strongly support the need for registration trials, facilitated by a ctDNA testing programme.

Our study did not show benefit from increasing the dose of fulvestrant in patients with ESR1 mutations in ctDNA. Previous research has suggested that fulvestrant at standard doses does not maximally inhibit or degrade mutated ESR1,a and we assessed whether more frequent administration of fulvestrant would increase therapeutic utility. Although exposure was increased in later cycles, this was insufficient to enhance activity, with the response rate remaining similar to that previously reported.8,9 We note however that our study recruited a heavily pretreated population, and this might have reduced the activity of fulvestrant. More potent oestrogen receptor inhibitors, such as novel oral oestrogen receptor degraders and modulators, are also likely to be required.21 We found that patients with Tyr537Ser ESR1 mutations were no less sensitive to fulvestrant than those with other ESR1 mutations, and that ESR1 mutations were frequently subclonal, with detection of ESR1 mutations in ctDNA that were not present in contemporaneous single site tissue biopsies, reflecting the limited sampling of single site tissue biopsies. Fulvestrant activity was similar in patients with and without ESR1 mutations in tissue sequencing.

Our study has limitations. Inclusion of relatively heavily pretreated patients might reduce activity of the targeted drugs, especially in cohort A, and future ctDNA selection trials might benefit from more restrictive entry criteria. The study was designed to assess the activity of therapies against specific genomic events, but it did not target PIK3CA mutations; and as a result relatively few of the patients registered to the trial had a response to therapy (17 [1·6%] of 1051 patients). However, mutation-directed therapy with alpelisib is now approved to target PIK3CA mutations, and our study shows the clinical validity of using ctDNA to direct therapy. Cohort D was designed as a basket cohort from the outset, to explore the activity of capivasertib against different AKT pathway activating mutations. Only cohort D allowed entry of patients with previous tissue sequencing results, as it was anticipated that ctDNA testing alone might not recruit sufficient patients. Although we identified low activity of capivasertib in PTEN-mutant cancers when used as a single agent, AKT inhibition in combination with paclitaxel chemotherapy might be efficacious in PTEN-mutant cancers.10,11 Capivasertib plus fulvestrant might be efficacious in endocrine-resistant oestrogen receptor-positive breast cancer without mutation selection, as shown in the FAKTION trial.10 It is not possible to robustly compare plasmaMATCH with FAKTION, as patients enrolled in plasmaMATCH had more previous lines of treatment, and AKT1 mutations were not assessed and would be few in number in FAKTION.11

In conclusion, we show that ctDNA testing, with the assays employed in this study, has sufficient accuracy for widespread adoption in routine clinical practice to identify patients with breast cancer who are suitable for licensed targeted therapies, such as PIK3CA-mutant breast cancer, with the transformative potential of efficient and rapid screening for clinical trials. A high proportion of patients with specific targeted mutations were able to enrol on the matching treatment cohort, with clinically important activity observed with therapies matched to AKT1 and HER2 mutations. With mutation-matching therapy now approved in breast cancer, with alpelisib for PIK3CA-mutant disease, ctDNA testing can be seen as a standard-of-care test for both common and rare targetable genetic events.

Contributors
NCT is the chief investigator, and AR is the coordinating investigator for the trial. JMB is the trials methodology lead within the Institute of Cancer Research Clinical Trials and Statistics Unit (ICR-CTSU) and provided oversight and guidance for trial management, statistics, and data interpretation throughout the trial. NCT, AR, and JMB were responsible for study design and acquired the funding for the trial. AMW, IRM, RDB, RR, and AR are all cohort clinical leads responsible for the clinical oversight and safety review and evaluation for a defined treatment cohort within the trial. NCT and AR wrote the first draft of the manuscript. LSK undertook statistical analyses and contributed to data interpretation. AMW, IRM, RDB, RR, PS, OO, JPB, MT, MCW, DR, DC, AS, MH, and KR are members of the plasmaMATCH trial management group, which contributed to study design, was responsible for oversight throughout the trial, and contributed to data interpretation and manuscript preparation. PS, OO, JPB, MT, JA, MCW, and DC were involved in recruitment and treatment of participants and contributed to data collection and manuscript preparation. HB and MH are responsible for the digital PCR ctDNA testing within the trial. CS is the trial operational lead responsible for the central management of the trial at ICR-CTSU. LM, SK, KW, and SM managed the study and data collection at ICR-CTSU. KR is a patient advocate member of the trial management group and provided guidance for trial documentation and reports. BK, HG, and RC analysed ctDNA results and did tissue sequencing. All authors reviewed and approved the manuscript.

Declaration of interests
NCT, AR, JMB, LSK, CS, LM, SK, KW, SM, HB, MH, BK, and RC report grants from Cancer Research UK, grants and non-financial support in the form of study drug provision from AstraZeneca and Puma Biotechnology, non-financial support in the form of ctDNA sequencing from Guardant Health, and provision of reagents from BioRad, during the conduct of the study. NCT also reports grants and personal fees from AstraZeneca, Pfizer, and Roche Genentech, personal fees from Bristol-Myers Squibb, Lilly, Merck Sharpe & Dohme, Novartis, Bicycle Therapeutics, Taiho Pharmaceutical, Zeno Pharmaceuticals, and Repare Therapeutics, and grants from BioRad, Clovis, Merck Sharpe and Dohme, and Guardant Health, outside the submitted work. BK also reports personal fees from Guardant Health outside the submitted work. AMW reports personal fees from Roche, personal fees and other support from Novartis, Pfizer, Lilly, Daiichi Sankyo, Merck Sharpe & Dohme, AstraZeneca, and AthenaRx, and other support from Seattle Genetics, Andrew Wardley, and Manchester Cancer Academy and Outreach Research and Innovation Group, outside the submitted work. IRM reports personal fees and non-financial support from Roche, Eli Lilly, and Eisai, and personal fees from Novartis, Pfizer, Daiichi Sankyo, Genomic Health, Pierre Fabre, and Merck Sharpe & Dohme, outside the submitted work. RDB reports grants from AstraZeneca and Roche Genentech, outside the submitted work. RR reports personal fees from Novartis, Eli Lilly, and Pfizer, personal fees and non-financial support
from Daichi Sankyo and G1 Therapeutics, and non-financial support from Roche and AstraZeneca, outside the submitted work. PS reports personal fees from Novartis, Eisaí, and Daichi Sankyo, outside the submitted work. OI reports grants and personal fees from Pfizer and Eisaí, personal fees from Roche Genentech and Tesaro, non-financial support from AstraZeneca, personal fees and non-financial support from Eli Lilly, and grants from Novartis, outside the submitted work. MT reports personal fees from Pfizer, Novartis, Roche, Vaccitech, Oxford Vaccines, Lilly, Astellas, Genomic Health, Eisaí, personal fees and non-financial support from Janssen, Bristol-Myers Squibb, and Ipsen, and non-financial support from EUSA Pharma, outside the submitted work. JA reports grants and personal fees from Eisaí and personal fees from Merck, outside the submitted work. MCW reports personal fees and non-financial support from Eisaí, Lilly, and Roche, and personal fees from Pfizer, Genomic Health, and Novartis, outside the submitted work. HB also reports personal fees from AstraZeneca outside the submitted work. MH also reports personal fees from Bristol-Myers Squibb, Boehringer Ingelheim, Roche Diagnostics, and Eli Lilly, during the conduct of the study. DR reports personal fees from Novartis, Pfizer, Roche, Daichi Sankyo, Lilly, and Genomic Health, non-financial support from Daichi Sankyo and Eisaí, and grants from Celgene, Roche, Biotheranostics, and RNA Diagnostics, outside the submitted work. DC reports other support from Novartis, AstraZeneca, Pfizer, Roche, Eli Lilly, Puma Biotechnology, Daichi Sankyo, Syntho, Seagen, Zymeworks, Elsevier, European Cancer Organisation, Celgene, Succint Medical Communications, Prima Biomed, Oncolytics Biotech, Celldex Therapeutics, San Antonio Breast Cancer Consortium, Highfield Communication, Samsung Bioepis, prIME Oncology, Merck Sharp & Dohme, RTI Health Solutions, and Eisai, outside the submitted work. DC also reports he was chief investigator for one of the trials included in the manuscript; all funding for that trial went to an institution other than his own; Roche, Pfizer, Amgen, and Cancer Research UK all supported the TACT2 trial by unrestricted educational grants (from the companies) or a clinical trials award (from Cancer Research UK) paid to the Institute of Cancer Research. AS reports grants from Ventana Roche, and Genomic Heath, personal fees from Daichi Sankyo, Hologic, Genomic Health, and Ventana Roche, outside the submitted work. JMB also reports grants and non-financial support from AstraZeneca, Novartis, Janssen Cilag, Merck Sharp & Dohme, Pfizer, Roche, and Clovis Oncology, and grants from Medivation, outside the submitted work. AR also reports personal fees from Roche, Pfizer, Novartis, Lilly, and Merck Sharp & Dohme, outside the submitted work. All other authors declare no competing interests.

Data sharing
De-identified individual participant data, together with a data dictionary defining each field in the dataset, will be made available to other researchers on request, subject to approval of a formal data access request in accordance with the ICR-CTSU data and sample access policy. Trial documentation including the protocol are available on request by contacting plasmaMATCH-icrctsu@icr.ac.uk. The ICR-CTSU supports the wider dissemination of information from the research it conducts, and increased cooperation between investigators. Trial data are collected, managed, stored, shared, and archived according to ICR-CTSU standard operating procedures to ensure the enduring quality, integrity, and utility of the data. Formal requests for data sharing are considered in line with ICR-CTSU procedures, with due regard given to funder and sponsor guidelines. Requests are to be made via a standard proforma describing the nature of the proposed research and extent of data requirements. Data recipients are required to enter a proforma describing the nature of the proposed research and extent of data requirements. Data recipients are required to enter a formal data sharing agreement, which describes the conditions for data release and requirements for data transfer, storage, archiving, publication, and intellectual property. Requests are reviewed by the trial management group in terms of scientific merit and ethical considerations including patient consent. Data sharing is undertaken if proposed projects have a sound scientific or patient benefit rationale, as agreed by the trial management group and approved by the trial steering committee, as required. Restrictions relating to patient confidentiality and consent will be lessened by aggregating and anonymising identifiable patient data. Additionally, all indirect identifiers that may lead to deductive disclosures will be removed in line with Cancer Research UK data sharing guidelines. Additional documents may be shared if approved by the trial management group and trial steering committee—eg, statistical analysis plan and informed consent form.

Acknowledgments
plasmaMATCH is funded by Cancer Research UK (C1491/A25351) with additional support from AstraZeneca, Puma Biotechnology, Guardant Health, and BioRad. Grateful thanks to all trial participants and their families. We thank Breast Cancer Now for funding this work as part of Programme Funding to the Breast Cancer Now Toby Robbins Research Centre. Thanks also to staff at participating centres, the ICR-CTSU trial team, the staff at central laboratories, and Syed Haider and his team in the Breast Cancer Now Toby Robbins Research Centre Bioinformatics Core Facility for bioinformatics support. plasmaMATCH is co-sponsored by the Institute of Cancer Research and the Royal Marsden National Health Service Foundation Trust. ICR-CTSU is supported by the Cancer Research UK core grant (C1491/A25351). plasmaMATCH is supported by the National Institute for Health Research (NIHR) Manchester Clinical Research Facility at the Christie Hospital, Manchester, UK, and by the Cancer Research UK Cambridge Centre, the Cambridge NIHR Biomedical Research Centre, and the Cambridge Experimental Cancer Medicine Centre, Cambridge, UK. plasmaMATCH is supported at participating sites in England by the NIHR Clinical Research Network, in Scotland by the Chief Scientist Office, and in Wales by Health and Care Research Wales. This study represents independent research supported by the NIHR Biomedical Research Centre at the Royal Marsden National Health Service Foundation Trust and the Institute of Cancer Research, London, UK. The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care. The authors also acknowledge past and present colleagues on the plasmaMATCH trial management group, the independent data monitoring committee, and the trial steering committee, who provided oversight of the trial (appendix p 3).

References
1 André F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med 2019; 380: 1929–40.
2 Bertucci F, Nig CKY, Patounakis A, et al. Genomic characterization of metastatic breast cancers. Nature 2019; 569: 560–64.
3 Razavi P, Chang MT, Xu G, et al. The genomic landscape of endocrine-resistant advanced breast cancers. Cancer Cell 2018; 34: 427–38.
4 Hyman DM, Pihan-Paul SA, Won H, et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature 2018; 554: 189–94.
5 Hyman DM, Smyth LM, Donoghue MTA, et al. AKT inhibition in solid tumors with AKT1 mutations. J Clin Oncol 2017; 35: 2251–59.
6 Nayar U, Cohen O, Kapstad C, et al. Acquired HER2 mutations in ER metastatic breast cancer confer resistance to estrogen receptor-directed therapies. Nat Genet 2019; 51: 207–16.
7 Pearson A, Proszek P, Pascual J, et al. Inactivating NFI mutations are enriched in advanced breast cancer and contribute to endocrine therapy resistance. Clin Cancer Res 2020; 26: 608–22.
8 De Mattos-Arruda L, Sammut S-J, Ross EM, et al. The genomic and immune landscapes of lethal metastatic breast cancer. Cell Rep 2019; 27: 2600–708.
9 O’Leary B, Cutts RJ, Liu Y, et al. The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discov 2018; 8: 1390–403.
10 Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013; 368: 1199–209.
11 Kim SB, Dent R, Wongchenko MJ, Singel SM, Baselga J. Concordance between plasma-based and tissue-based next-generation sequencing in LOTUS. Lancet Oncol 2017; 18: e638.
12 Torga G, Pienta KJ. Patient-paired sample congruence between 2 commercial liquid biopsy tests. JAMA Oncol 2018; 4: 868–70.
13 Stetson D, Ahmed A, Xu X, et al. Orthogonal comparison of four plasma NGS tests with tumor suggests technical factors are a major source of assay discordance. JCO Precis Oncol 2019; 3: 1–9.
14 Merker JD, Oxnard GR, Compton C, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. J Clin Oncol 2018; 36: 1631–41.
15 André F, Bachelot T, Commo F, et al. Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER). Lancet Oncol 2014; 15: 267–74.
16 Lee J, Kim ST, Kim K, et al. Tumor genomic profiling guides patients with metastatic gastric cancer to targeted treatment: the VICTORY umbrella trial. Cancer Discov 2019; 9: 1388–405.
17 Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013; 31: 1023–31.
18 Toy W, Shen Y, Won H, et al. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. Nat Genet 2013; 45: 1439–45.
19 Turner NC, Ro J, André F, et al. Palbociclib in hormone-receptor-positive advanced breast cancer. N Engl J Med 2015; 373: 209–19.
20 Johnston SR, Kilburn LS, Ellis P, et al. Fulvestrant plus anastrozole or placebo versus exemestane alone after progression on hormone-receptor-positive locally advanced or metastatic breast cancer (SoFEA): a composite, multicentre, phase 3 randomised trial. Lancet Oncol 2013; 14: 989–98.
21 Toy W, Weir H, Razavi P, et al. Activating ESR1 mutations differentially affect the efficacy of ER antagonists. Cancer Discov 2017; 7: 277–85.
22 Schmid P, Abraham J, Chan S, et al. Capivasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer: the PAKT trial. J Clin Oncol 2020; 38: 423–33.
23 Kim SB, Dent R, Im SA, et al. Ipatasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (LOTUS): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol 2017; 18: 1360–72.
24 Jones RH, Casbard A, Carucci M, et al. Fulvestrant plus capivasertib versus placebo after relapse or progression on an aromatase inhibitor in metastatic, oestrogen receptor-positive breast cancer (FAKTION): a multicentre, randomised, controlled, phase 2 trial. Lancet Oncol 2020; 21: 345–57.