**Directing therapy with circulating tumour DNA analysis in advanced breast cancer: The multi-cohort plasmaMATCH platform trial**

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**Abstract**

Background

Circulating tumour DNA (ctDNA) testing may provide a contemporary assessment of the genomic profile of advanced cancer, without need to repeat tumour biopsy. The plasmaMATCH trial was designed to assess the accuracy of ctDNA testing in a large prospective study in advanced breast cancer, and the ability of ctDNA testing to select patients for mutation-directed therapy.

Methods

plasmaMATCH is an open label, multi-centre, multi-cohort platform trial of ctDNA testing in ~1000 patients of at least 18 years of age with advanced breast cancer and ECOG performance status 0-1. Patients had at least one prior line of treatment for advanced breast cancer and/or relapsed within 12 months of (neo)adjuvant chemotherapy. Patients with HER2 amplified (HER2-positive) breast cancer had at least two prior lines of advanced HER2 targeted therapy. Patients were recruited into four parallel treatment cohorts matched to mutations identified in ctDNA: A:*ESR1* mutation (extended-dose fulvestrant – 500mg IM on Cycle 1 Day 1, 8 and 15 and Cycle 2 onwards Day 1 and 15 on a 28-day cycle) , B:*ERBB2* mutation (neratinib – 240mg orally QD – and if ER-positive with standard dose fulvestrant 500mg IM on Cycle 1 Day 1 and 15 and Cycle 2 onwards Day 1 on a 28-day cycle), C:*AKT1* mutation (ER-positive cancer –capivasertib 400mg orally BID 4 days on / 3 days off on a 28-day cycle with standard dosing fulvestrant), ctDNA testing was conducted with digital PCR and error-corrected targeted sequencing. Tumour sequencing from an advanced disease biopsy was conducted retrospectively, not influencing cohort entry. Cohort D was an *AKT* activation basket (*AKT1* mutation ER-negative cancer or *PTEN* mutation –capivasertib 480mg orally BID 4 days on / 3 days off on a 28-day cycle) that recruited both on the basis of ctDNA testing and external tissue sequencing. Treatments were given until progression. Each cohort had a phase II single-arm design with primary endpoint of confirmed objective response rate using RECISTv1.1 in an evaluable patient population (patients with measurable disease as per RECIST at baseline and at least one on-treatment assessment). For Cohort A ≥13/78 responses were required to infer efficacy and similarly ≥3/16 responses were required for Cohorts B-D. Recruitment to cohorts A-D is complete and long-term follow-up continues. Trial registration NCT03182634; EudraCT2015-003735-36; ISRCTN16945804.

Findings

Between December 21, 2016 and April 26, 2019, 1051 patients registered, with ctDNA testing results available for 1034 patients. Agreement between ctDNA digital PCR and targeted sequencing was 95.5%-99.4% (n=800, kappa 0.89-0.93). ctDNA testing had 93-98% sensitivity for mutations identified in tissue sequencing. 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. In patients with *ERBB2* mutations, response rate with neratinib, plus fulvestrant in ER-positive cancer, was 25.0% (5/20, 95%CI 8.7-49.1%). In patients with *AKT1* mutations and ER-positive cancer, response rate with capivasertib plus fulvestrant was 22.2% (4/18, 95%CI 6.4-47.6%). Cohorts A and D did not reach the target number of responses. Response rate of *ESR1* mutations with extended-dose fulvestrant was 8.1% (6/74, 95%CI 3.0-16.8%). In patients with *AKT* activation basket mutations the response rate was 10.5% (2/19, 95%CI 1.3-33.1%). In patients with *AKT1* mutations and ER-negative cancer, 33.3% (2/6, 95%CI,4.3-77.7) responded to capivasertib, although 0/13 patients with *PTEN* mutation responded. The most common grade 3-4 adverse events were: Cohort A: hypertension 12.5% (10/80); Cohort B: diarrhoea – 25.0% (4/20) and hypertension 15.0% (3/20); Cohort C: fatigue – 22.2% (4/18), rash - 16.7% (3/18), diarrhoea - 11.1% (2/18) and hyperglycaemia - 11.1% (2/18); Cohort D: rash - 26.3% (5/19), hypertension – 10.5% (2/19), transaminase increase 10.5% (2/19) and vomiting 10.5% (2/19). A total of 18 serious adverse reactions in 11 patients were reported and 1 treatment-related death (cohort C).

Interpretation

ctDNA testing offers accurate, rapid genotyping that enables the selection of patients for mutation-directed therapies, with sufficient clinical validity for adoption into routine clinical practice. Our results demonstrate clinically relevant activity of targeted therapies against rare *ERBB2* and *AKT1* mutations, confirming these mutations may be targetable for breast cancer treatment.

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**Research in context**

*Evidence before this study*

We searched PubMed on Jun 11, 2020, for clinical trials published between 2000 and 2019 with the terms “circulating tumor DNA” AND “cell free DNA” AND “plasma DNA” AND “liquid biopsy” and “ctDNA” with no restriction on language, and identified 212 results. Circulating tumour DNA analysis in multiple retrospective trials has been shown to accurately genotype mutations found in the tumour Circulating tumour DNA analysis therefore has the potential to transform the selection of targeted therapies for patients with advanced cancer. Yet there has been uncertainty about validity of ctDNA testing in routine practice, as there have been few large prospective studies to assess the accuracy and utility of ctDNA testing. The TARGET trial reported in 2019 a potential role for ctDNA testing in 100 patients with advanced cancers in an early phase clinical trial setting. In addition, sensitivity has not been perfect in prior 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. The ASCO-CAP guidelines committee on ctDNA analysis concluded in 2018 the lack of prospective trials was one of the major weaknesses in evidence on bringing ctDNA testing through to routine practice, with a need for trials that recruited patients solely on the basis of ctDNA testing without prior tissue testing.

*Added value of this study*

plasmaMATCH represents to our knowledge the first large prospective, multi-centre study assessing the utility of circulating tumour DNA analysis to direct therapy in patients with advanced breast cancer. It recruited 1051 patients for ctDNA testing, from both academic and general hospitals, testing with two orthogonal ctDNA analysis techniques. Very high agreement between ctDNA assays is demonstrated, and very high sensitivity for mutations identified in tissue sequencing, especially with contemporaneous biopsies. Mutation directed therapy with alpelisib is approved to target *PIK3CA* mutations, and our study demonstrates the clinical validity of using ctDNA to direct therapy.

Patients with rare, potentially-targetable mutations in *ERBB2* and *AKT1* in ctDNA, had clinically important responses with the *ERBB2* inhibitor neratinib and *AKT* inhibitor capivasertib respectively, similar to activity seen in prior tissue sequencing directed trials. These findings confirm that these mutations are targetable for breast cancer therapy, and demonstrate the validity and utility of using ctDNA testing to screen patients for rare mutations.

*Implications of all the available evidence*

These findings demonstrate 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 questions the need to conduct reflex tissue testing for negative ctDNA results, within the pre-treated metastatic breast cancer patient population recruited. This study also demonstrates 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.

**Introduction**

Multiple tumour mutations are potentially targetable for advanced breast cancer treatment. Some of these mutations are common, such as activating *PIK3CA* mutations targetable with PI3 kinase inhibitors including the recently approved alpelisib1. Other potentially targetable mutations, such as *ERBB2* and *AKT1* mutations, are rare genetic events, occurring in approximately 5% of advanced breast cancer patients2,3. Nonetheless they represent attractive therapeutic opportunities, with early phase studies based on tissue genotyping suggesting high response rates with matched targeted therapies4,5. Frequently, mutations in the estrogen receptor (*ESR1)*, *ERBB2* and *AKT1* may be acquired in advanced disease*,* not being detectable in the archival primary2,6,7. Therefore, archival primary tissue cannot be assumed to be representative of advanced disease genomic profile.

Mutation analysis can be obtained from genomic analysis of tissue-based biopsies from metastatic disease, however this process is invasive and potentially limited by tumour heterogeneity and temporal tumour evolution8,9. In addition, mutations may not be present at the time of relapse, and only later develop during treatment for advanced cancer, posing a clinical challenge in regards to acquisition of longitudinal tissue biopsies. Highly sensitive assays have recently been developed to analyse circulating tumour DNA (ctDNA), which is released into the plasma in small quantities as cancer cells die, providing the opportunity for a scalable non-invasive approach to profile tumours for somatic mutations10. Retrospective studies show high agreement between ctDNA analysis and tumour-tissue based analysis in patients with advanced cancer11. Yet previous prospective comparison studies between commercially available ctDNA assays have suggested the potential for substantial discordance in ctDNA testing results12,13, raising concerns over whether ctDNA testing is ready for widespread clinical adoption14.

plasmaMATCH is a platform trial designed to assess the clinical validity of ctDNA testing in a prospective multi-centre study, and to investigate the clinical utility of using ctDNA to select patients for targeted therapies without prior tissue testing.

**Methods**

**Study design and patients**

plasmaMATCH is a multiple parallel cohort, open-label, non-randomised, multi-centre, phase IIa clinical trial platform. The study was designed to recruit advanced breast cancer patients to undergo ctDNA testing for potentially targetable mutations, enabling entry into one of four parallel treatment cohorts with therapies matched to mutations identified in ctDNA (appendix p17). A fifth cohort (E) recruiting patients with triple negative breast cancer (TNBC) with no actionable mutation to receive olaparib plus the *ATR* inhibitor AZD6738 is ongoing and will be reported separately. Eligible patients were age ≥18 years had an ECOG performance status of 0/1, expected life expectancy of >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 had disease progression by radiological or clinical assessment at registration (with radiological confirmation required prior to treatment cohort entry), and had completed at least one prior line of treatment for advanced breast cancer and/or relapsed within 12 months of (neo)adjuvant chemotherapy. Patients with *HER2* amplified (HER2-positive) breast cancer had at least two prior lines of HER2 targeted therapy in the advanced setting (or one line if no further HER2 targeted therapies available). An approved protocol amendment implemented on February 19, 2018, after 515 patients had been recruited, required a maximum of two prior lines of chemotherapy, antibody‐drug conjugate or immunotherapy.

The study was co-sponsored by The Institute of Cancer Research and the Royal Marsden NHS Foundation Trust and approved by a Research Ethics Committee (16/SC/0271). All participants gave written informed consent prior to registration for ctDNA testing, and again prior to treatment cohort entry. Safety and efficacy data were reviewed regularly by an Independent Data Monitoring Committee (IDMC). Trial oversight was provided by an independent Trial Steering Committee.

**Procedures**

ctDNA testing was conducted with two technologies: digital droplet PCR at a central laboratory in the National Institute for Health Research (NIHR) Centre for Molecular Pathology at Royal Marsden and Institute of Cancer Research prospectively in all patients, for mutations in *PIK3CA, ESR1, ERBB2* and *AKT1*. From July 10, 2018(after recruitment of 680 patients)prospective testing also includederror corrected targeted sequencing with Guardant360® (Guardant Health) for a panel of 73 genes including *PIK3CA, ESR1, ERBB2, AKT1 and PTEN*, with retrospective sequencing performed for previously enrolled patients. For comparison with ctDNA results, tumour tissue sequencing using advanced disease tissue biopsies was conducted retrospectively for patients who entered a treatment cohort (appendix p5). 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 III studies of treatments for *PIK3CA* mutant breast cancer were ongoing when plasmaMATCH started recruitment1. In cohort A, *ESR1* mutations were determined to be clonally dominant orsub-clonal for exploratory analysis, with a clonally dominant mutation indicating a summed *ESR1* allele fraction ≥50% of maximum allele fraction detected in the sample by targeted sequencing to correct for variations in the purity of ctDNA in plasma DNA (appendix p5).

Investigators at UK hospitals registered eligible patients with the ICR Clinical Trials and Statistics Unit (ICR-CTSU) for ctDNA testing. Those with potentially actionable mutations identified in ctDNA testing (*ESR1*, *ERBB2*, *AKT1* or *PTEN*) were offered entry into one of four parallel treatment cohorts (appendix p17). A positive result by either assay was sufficient for cohort entry. If more than one mutation was identified, entry to Cohorts B-D took preference to A.

* Cohort A: *ESR1* mutation *–* extended-dose 500mg fulvestrant (selective estrogen receptor down-regulator) administered intramuscularly (IM) on Cycle 1 Days 1, 8 and 15 and Cycle 2 onwards Days 1 and 15 on a 28-day cycle. Pharmacokinetic (PK) analysis samples were collected pre-dose on Cycles 2-4 and compared to a historical population (popPK) model for standard-dose fulvestrant.
* Cohort B: *ERBB2* mutation – 240mg neratinib (irreversible pan-HERtyrosine kinase inhibitor) orally once daily on a continuous schedule. In patients with ER-positive breast cancer this was administered with fulvestrant 500mg IM at standard dosing (Cycle 1 Days 1 and 15 and Cycle 2 onwards Day 1 on a 28-day cycle).
* Cohort C: *AKT1* mutationin patients with ER-positive breast cancer –400mgcapivasertib (selective *AKT* inhibitor) orally twice daily 4 days on-treatment followed by 3 days off-treatment on a 28-day cycle with standard dosing fulvestrant.
* Cohort D: *AKT* activation basket: mutations in *AKT1* with ER-negative breast cancer, or *PTEN* inactivating mutations or homozygous deletion (irrespective of ER status); full criteria described in Supplementary Methods, appendix p6. Patients with mutations identified in prior tumour sequencing conducted outside of plasmaMATCH were eligible for this cohort - 480mg capivasertib monotherapy administered orally twice daily on a 7 day schedule of 4 days on-treatment followed by 3 days off-treatment on a 28-day cycle.

In addition to the eligibility criteria for ctDNA testing, and if patients had a relevant actionable mutation, patients would not be eligible without adequate haematological, renal and hepatic function (adequate defined as ANC≥1000/mm3 (≥1.0x109/L), platelet count ≥100,000/mm3 (≥100x109/L), haemoglobin ≥9g/dL (≥90g/L), serum creatinine ≤1.5xULN, total bilirubin ≤1.5xULN, ALT and AST ≤3xULN or ≤5xULN in the presence of liver metastases) and for cohorts C and D if baseline glycosylated haemoglobin (HbA1c) was ≥8.0% (64mmol/mol), or fasting plasma glucose ≥7.0mmol/L (126mg/dL), or if a patient had poorly controlled diabetes mellitus. Clinically significant or uncontrolled cardiac disease and QTc prolongation (>470ms) were exclusion criteria for Cohort B.

Once enrolled into a cohort, treatment was given until progression, unacceptable toxicity, or pregnancy. Participants could also discontinue from trial treatment at any time at their own request or discontinued at the discretion of the treating clinician. Within each cohort, dose modifications were permitted for patients experiencing toxicities related to treatment (appendix p8)

Patients underwent CT or MRI scan and bone scan at baseline, with CT or MRI scan repeated 8 weekly until 32 weeks, 12 weekly thereafter for disease evaluation using RECIST v1.1. There was no independent central review of disease outcome. Laboratory assessments, adverse event recording and vital signs were performed at least every 4 weeks. Toxicity was assessed using National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4. Coding was done by use of the Medical Dictionary for Regulatory Activities (version 22). Patients were eligible for cohort entry with detection of a mutation at any allele fraction, and also clonal dominance was not considered in eligibility.

**Outcomes**

The primary endpoint for Cohorts A–D was confirmed objective response rate defined as a confirmed complete response (CR) or partial response (PR) at any point during trial treatment according to Response Evaluation Criteria in Solid Tumours (RECIST) criteria, version 1.1. Secondary endpoints included duration of response (defined as time from the first documentation of CR or PR until date of progression or last date of follow-up), clinical benefit rate (defined as CR, PR or stable disease>6 months during trial treatment), progression-free survival (PFS, defined as time from cohort entry to first date of either confirmed progressive disease according to RECIST criteria or death from any cause. Patients who were alive and progression-free were censored at date of last follow-up. Patients who had non-RECIST confirmed progression (e.g. clinical progression or radiologically confirmed but lesions not measured according to RECIST) were censored at their date of progression), safety, 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 (Cohorts A and B). Exploratory endpoints included confirmed response rate in clonally dominant *versus* sub-clonal mutations in cohort A.**Statistical analysis**

All cohorts used a single stage A’Hern design with α=0.05, power 80%. 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 efficacy. This was an approved amendment (on 1 May 2018) to the original two-stage design to account for the ctDNA testing detecting sub-clonal mutations as well as clonal mutations where the response rate was expected to be lower (see appendix p6). 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 whilst 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 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 and clinical benefit rate were conducted in an evaluable population defined as those patients with measurable disease as per RECIST at baseline and at least one on-treatment assessment; patients who stopped treatment due to intolerable toxicity or death without having a scan after baseline were evaluable and recorded as non-responders. Proportions and two-sided 95%CI for estimation purposes were reported overall for each cohort and, in pre-specified subgroup analyses, by clonally dominant versus sub-clonal mutations, compared using a Fisher’s exact test, and for Cohort B separately looking at the HR+, HER2- subgroup and for Cohort D the *AKT1* and *PTEN* mutations. 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 (i.e. alpha 5%, one-sided), proportions and two-sided 90%CI are reported in the supplementary material (appendix p16). PFS used an intention-to-treat population. Kaplan Meier curves were plotted and median PFS is reported with IQR for each cohort. The safety population included all patients who had at least one dose of treatment and treatment emergent adverse events where >10% patients reported any grade or any patients reporting grade ≥3 were presented. In addition, PK was reported as percentage change from approved 500mg popPK model for standard 500mg fulvestrant dose in Cohort A only. Safety was assessed on the basis of the incidence of adverse events.

Analyses used a database snapshot taken on November 6, 2019. Where reported, p-values <0.05 are deemed statistically significant. All analyses were conducted using Stata (version 15.1). This study is registered with ClinicalTrials.gov, number NCT03182634; the European Clinical Trials database, number EudraCT2015-003735-36; and the ISRCTN registry, number ISRCTN16945804.

**Role of the funding source**

This study was funded by Cancer Research UK, AstraZeneca, and Puma Biotechnology with support from BioRad and Guardant Health. The funding sources had no role in the study design, collection, analysis, interpretation of data 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 paper for publication. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

**Results**

Between December 21, 2016 and April 26, 2019, 1051 patients from 18 UK hospitals were registered into the study (1044 via ctDNA screening, 7 via prior tumour sequencing, Figure 1 and appendix p17) with ctDNA results available for 99.0% patients (1034/1044); Digital PCR results in 1025 patients and targeted sequencing in 800 patients (364 prospective and 436 retrospective). The median time from blood draw to ctDNA results was 13 days for digital PCR (inter-quartile range, IQR:11-15) and 10 days for sequencing (IQR:8-11). Patients had had a median of 1 (IQR:0-2) prior lines of chemotherapy, and a median of 2 (IQR:1-3) prior lines of systemic therapy (Table 1).

A somatic mutation was detected in 92.9% (743/800) of patients with ctDNA targeted sequencing results. *ESR1* mutations were found almost exclusively in hormone receptor positive (HR-positive) breast cancer (appendix p18). *ERBB2* mutations were least common in TNBC, whilst *AKT1* mutations were found at similar frequency in HR-positive HER2-negative breast cancer and TNBC. *ESR1* mutations were found at lower average allele fractions than other mutations (appendix p17), and were frequently polyclonal (appendix p18).

Gene-level agreement for mutation calling between ctDNA digital PCR and targeted sequencing was 95.5%-99.4% (kappa 0.89-0.93, Figure 1). Individual mutation agreements were also high (appendix p19). An advanced tissue biopsy was sequenced retrospectively for 77 patients who entered a cohort. Sensitivity (or percent positive agreement reflecting the lack of gold standard) for digital PCR was 93% (95%CI 83.4-97.5, appendix p17) rising to 98% (95%CI 86.8-99.9) in patients with contemporaneous biopsies (appendix p17). In contrast, digital PCR sensitivity in patients with time discordant biopsies was 85% (95%CI 66.3-95.8, appendix p20). Sensitivity for targeted sequencing was 95% overall (95%CI 87.1-99.0) and 100% contemporaneous (95%CI 91.6-100.0, appendix p21). Specificity (or percent negative agreement reflecting the lack of gold standard) was high, varying by gene (appendix p17).

Actionable mutations were identified in 357 (34.5%) of 1034 patients and 136 of these entered one of the 5 available cohorts. In those that 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 due to cohort A being suspended (while the protocol amendment to change the design was ongoing) or closed (appendix p7).

Of the 222 patients with an *ESR1* mutation in ctDNA whilst Cohort A was open to recruitment, 84 (37.8%) were enrolled (details in Figure 1); all had ER-positive cancers, three (3.6%) were HER2-positive (over-expressed/amplified), and 78 (92.9%) had visceral metastases (Table 1). The most common *ESR1* mutations detected in plasma were D538G (53.6%,45/84), Y537S (36.9%,31/84), E380Q (34.5%,29/84). 74 patients were evaluable for response, since four patients did not start treatment and six did not have on-treatment RECIST assessable imaging. Six patients had a confirmed response giving a response rate of 8.1% (95%CI,3.0-16.8%, Figure 2) with a median duration of response of 7.0 months (IQR: 3.7-8.3) and four patients continuing on treatment. Clinical benefit rate was 16.2% (95%CI,8.7-26.6%;12/74 patients). A total of 69/84 (82.1%) patients have had a RECIST-confirmed progression event or death. Median PFS in all 84 patients was 2.2 months (IQR: 1.7-5.3, appendix p22). In a pre-planned exploratory analysis response rate in those with clonally dominant *ESR1* mutations was 12.2% (5/41, 95%CI,4.1-26.2%) with no responses (0/27, 95%CI,0-12.8%) in those with sub-clonal mutations (p=0.15, 40% subclonal); six patients had unknown clonality. The most common grade 3/4 adverse event was hypertension (10/80, 12.5%) (appendix p9). One patient had a serious adverse reaction due to grade 3 superior sagital sinus thrombosis. There were no treatment-related deaths and 41/84 (48.8%) deaths reported (38 breast cancer and 3 unknown cause). The main reason for treatment discontinuation was disease progression (72/76 (94.7%), (appendix p8). Two patients had frequency reductions of fulvestrant, one due to bone pain and increased fatigue, the other due to low BMI and pain due to injections. 29 patients did not have a day 15 injection given on at least 1 occasion, with the main reason was disease progression. Ten delays to treatment were reported in 9 patients, with the main reason being patient choice. The median relative dose intensity in patients starting treatment was 100.0% (IQR:96.7-100.0, min-max:33.3-103.8). Pharmacokinetic analysis was consistent with elevated fulvestrant exposure compared with standard 500mg PopPk fulvestrant (appendix p10).

Of the 36 patients with *ERBB2* mutation in ctDNA, 21 (58.3%) enrolled in Cohort B (Figure 1); 18 (85.7%) had ER-positive cancers, three (14.3%) were HER2-positive (over-expressed/amplified), and 18 (85.7%) had visceral metastases (Table 1). The most common *ERBB2* mutations detected in baseline plasma were L755S (47.6%, 10/21), V777L (19.0%, 4/21), S310F (14.3%, 3/21). 20 patients were evaluable for response, as one patient did not start treatment. Confirmed response rate was 25.0% (5/20, 95%CI,8.7-49.1%), an additional three patients had unconfirmed partial responses (Figure 3). Four of the protocol specified first 16 evaluable patients responded (RR=25.0%, 95%CI,7.3-52.4%). One patient had a complete response, ongoing at 29 months duration. Median duration of response was 5.7 months (IQR: 3.7-9.7 months) with three patients continuing on-treatment. Clinical benefit rate was 45.0% (95%CI,23.1-68.5%;9/20 patients). A total of 16/21 (76.2%) patients have had a RECIST-confirmed progression event or death. Median PFS was 5.4 months (IQR: 3.4-9.1, appendix p23). In the subset of patients with HR-positive HER2-negative breast cancer treated with neratinib and fulvestrant there was a 23.5% response rate (95%CI,6.8-49.9;4/17, Figure 3). In an exploratory analysis, 16% (3/19) *ERBB2* mutations were subclonal (appendix p24). The most common grade 3/4 adverse events were diarrhoea (4/20, 20.0%) and hypertension (3/20, 15.0%) (appendix p11). Four serious adverse reactions were reported in three patients (appendix p11). There were no treatment-related deaths and 13/21 (61.9%) deaths reported (12 breast cancer and 1 unknown cause). The main reason for treatment discontinuation was disease progression (16/17 (94.1%), appendix p8). All 17 patients with ER-positive breast cancer who started treatment received all doses of fulvestrant. Neratinib dose was reduced to 160mg for 6/20 patients (30.0%), of which 1 patient also reduced to 120mg following a reduction to 160mg, with the main reason being adverse events (back pain, rash or diarrhoea). The median relative dose intensity of patients starting treatment was 92.4% (IQR:83.7-98.9, min-max:58.7-100) for neratinib and 100.0% (IQR:100.0-100.2, min-max:96.0-101.9) for fulvestrant.

Of the 30 patients with an *AKT1* mutation in ctDNA and ER-positive cancers, 18 (60.0%) enrolled in Cohort C; one (5.6%) was HER2-positive (over-expressed/amplified), and 17 (94.4%) had visceral metastases (Table 1). The most common mutation detected was *AKT1* E17K in 17 patients, with *AKT1* L52R detected in one patient. All patients were evaluable, with a confirmed response rate of 22.2% (4/18, 95%CI,6.4-47.6%), and an additional four patients had unconfirmed partial responses (Figure 4A). Three of the protocol specified first 16 evaluable patients responded (RR=18.8%, 95%CI,4.0-45.6%). Median duration of response was 7.5 months (IQR:4.1-9.8%) with four patients continuing on-treatment. A total of 12/18 (66.7%) patients have had a RECIST-confirmed progression event or death. Median PFS was 10.2 months (IQR 3.2-18.2, appendix p25). In an exploratory analysis, 23% (4/17) *AKT1* mutations were subclonal (appendix p24). The most common grade 3/4 adverse events were fatigue (22.2%), rash (16.7%), diarrhoea (11.1%) and hyperglycaemia (11.1%) (appendix p13). Eight serious adverse reactions were reported in four patients (appendix p13). There was one treatment-related death caused by grade 4 dyspnoea and 6/18 (33.3%) breast cacner deaths reported. The main reason for treatment discontinuation was disease progression (11/14 (84.6%), appendix p8). All 18 patients received all doses of fulvestrant. 10 dose reductions of capivasertib to (7 to 320mg, with a further 3 reducing further to 240mg) were made in 7/18 patients (38.9%), with the main reason being adverse events (diarrhoea, nausea, fatigue and rash). The median relative dose intensity was 87.5% (IQR:70.3-99.4, min-max:25.0-100.7) for capivasertib and 98.6% (IQR:96.8-100.0, min-max:93.8-101.9) for fulvestrant.

In total 19 patients were recruited in Cohort D, 12 following ctDNA testing and seven based on tumour testing; 13 (68.4%) patients had ER-positive cancers, all were HER2-negative, and 14 (73.7%) had visceral metastases (Table 1). The mutations detected were *AKT1* E17K (five patients), *AKT1* L52R (one), *PTEN* inactivating mutation (12), and PTEN homozygous deletion (one). All patients were evaluable, with a confirmed response rate of 10.5% (2/19, 95%CI,1.3-33.1, Figure 4B). Median duration of response was 3.9 months (IQR:3.7-4.2 months) with one patient continuing on treatment. Two of the six patients with *AKT1* mutations responded (33.3%, 95%CI,4.3-77.7) with two further unconfirmed responses. There were no responses in patients with *PTEN* genomic alterations (0/13). Clinical benefit rate was 10.5% (95%CI,1.3-33.3%;2/19 patients). A total of 13/19 (68.4%) patients have had a RECIST-confirmed progression event or death. Median PFS was 3.4 months (IQR:1.8-5.5, appendix p26). The most common grade 3/4 adverse events were rash (26.3%), hypertension (10.5%), transaminase increase (10.5%) and vomiting (10.5%) (appendix p15). Four serious adverse reactions were reported in three patients (appendix p15). There were no treatment-related deaths and 10/19 (52.6%) breast cancer deaths reported. The main reason for treatment discontinuation was disease progression (15/17 (83.3%), appendix p8). 5 dose reductions of capivasertib (4 to 400mg and 1 to 320mg) were made in 5/19 patients (26.3%), with the main reason being adverse efects (rash, diarrhoea, vomiting, hyperglycaemia and alkaline phosphatase and GGT raised). The median relative dose intensity of capivasertib was 93.8% (IQR:62.5-100.0, min-max:42.2-101.5).

In exploratory unplanned analysis the response rates in cohorts A-D did not vary by *PIK3CA* or *TP53* co-mutational status (appendix p27 and 28).).

**Discussion**

Within this large combined prospective ctDNA testing and multi-cohort platform trial in advanced breast cancer we have shown that ctDNA testing was highly accurate, with high agreement between different ctDNA testing techniques, with high sensitivity for mutations identified in tissue advanced cancer biopsies. ctDNA testing identified patients with rare targetable mutations and these patients were recruited onto matching targeted therapies without confirmatory tumour testing, with activity comparable to prior studies based on tumour tissue testing4,5. We demonstrated the ability to run a large national ctDNA screening-based platform study in advanced breast cancer, enrolling over 1000 patients from across the UK within less than three years, with a dynamic trial platform design evaluating multiple targeted treatment options simultaneously.

The availability and accuracy of ctDNA testing demonstrated in this study compares favourably with tissue-based mutation testing. Nearly all patients (98.9%) received a result from ctDNA testing, contrasting with prior tumour sequencing studies where results were typically received in only 70-90% patients15,16. In addition, prior tumour sequencing studies generally only included patients with biopsiable disease, which is not a constraint for ctDNA testing. Results were received relatively quickly after blood draw, compared to tissue-based testing, and this resulted in 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 sequencing17. Discordance between ctDNA results was still observed for patients at low allele frequency mutations (appendix p17), suggesting further potential for assay development. 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 copy number-based assessment. Digital PCR offered similar accuracy to sequencing, with substantial cost efficiency, although was limited to the specific mutations analysed. The academic clinical laboratory conducting the digital PCR assay achieved the trial target turnaround time of results within 14 days. A lower turnaround time could easily be achieved if required in clinical practice, representing a cost-efficient method of ctDNA analysis. Of the 1044 patients who underwent ctDNA testing, in total 533 had a potentially actionable mutation (51% - *PIK3CA, ESR1, ERBB2, AKT1, or PTEN)* indicating a potential value for ctDNA testing.

Our results confirm clinically relevant activity of targeted therapies against rare activating mutations in breast cancer. In a prior phase I study with expansion cohort of neratinib, in *ERBB2* mutant breast cancer identified in tissue, there was a 32% unconfirmed response rate at eight weeks4. Here, neratinib in *ERBB2* mutant breast cancer identified in ctDNA had comparable activity to that observed guided by tissue testing, with durable responses. Similarly, capivasertib had high activity in patients with ctDNA identified *AKT1* mutations, both in HR-positive cancer with fulvestrant and in HR-negative cancer as a single agent, again confirming results of a prior phase I study5. Confirming the high activity of these drugs against *ERBB2* and *AKT1* mutations, strongly suggest the need for registration trials, facilitated by a ctDNA testing program.

Our study did not show benefit from increasing the dose of fulvestrant in patients with *ESR1* mutations in ctDNA. Prior research has suggested that fulvestrant at standard doses does not maximally inhibit or degrade mutated *ESR1*18, and we assessed whether more frequent administration of fulvestrant would increase therapeutic utility. Although exposure was increased in later cycles (appendix p10) this was insufficient to enhance activity with the response rate similar to that previously reported19,20. We note however that this study recruited a heavily pre-treated population that may have reduced the effectiveness of fulvestrant (Table 1). More potent ER inhibitors, such as novel oral estrogen receptor degraders and modulators, are also likely required21. In this study patients with Y537S *ESR1* mutations were no less sensitive to fulvestrant than those with other *ESR1* mutations, *ESR1* mutations were frequently sub-clonal (Figure 2), with detection of *ESR1* mutations in ctDNA not present in contemporaneous single-site tissue biopsies (appendix p17), reflecting the limited sampling of single-site tissue biopsies. Efficacy was similar in patients with and without *ESR1* mutations in tissue sequencing (appendix p29).

Our study has limitations. Inclusion of relatively pre-treated patients may have limited efficacy, especially in cohort A, and future ctDNA selection trials may benefit from more restrictive entry criteria. The study was designed to assess the efficacy of therapies against specific genomic events, it did not target *PIK3CA* mutations1, and reflecting this design relatively few of the overall tested patients responded to therapy (17/1051). Cohort D was conceived as a basket cohort in plasmaMATCH from the outset, to explore the efficacy of capivasertib in different AKT activating mutations. Only cohort D allowed entry of patients with prior tissue sequencing results, as it was anticipated that ctDNA testing alone may not recruit sufficient patients. Although we identify low activity of capivasertib in *PTEN* mutant cancers as a single agent, AKT inhibition in combination with paclitaxel chemotherapy may be efficacious in *PTEN* mutant cancers22,23. Capivasertib plus fulvestrant may be efficacious in endocrine resistant ER-positive breast cancer without mutation selection in the FAKTION trial24. It is not possible to robustly compare plasmaMATCH with FAKTION, as plasmaMATCH patients had higher levels of prior treatment, and *AKT1* mutations were not assessed and would be few in number in FAKTION24.

In conclusion we show that ctDNA testing, with the assays employed in this study, has sufficient accuracy for widespread adoption in clinical practice to identify patients suitable for licensed targeted therapies in routine clinical practice, 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 enroll on the matching treatment cohort, with clinically important activity observed with therapies matched to *AKT1* and *ERBB2* mutations. With mutation matching therapy now approved in breast cancer, with alpelisib in *PIK3CA* mutant disease, ctDNA testing can be seen as a standard-of-care testing 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 the 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. NT 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 (TMG), 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 TMG and provided guidance for trial documentation and reports. BK, HG and RC analysed ctDNA results and conducted tissue sequencing. All authors reviewed and approved the manuscript.

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**Declaration of interests**

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AR also reports personal fees from Roche Products Limited, Pfizer, Novartis, Lilly and MSD outside the submitted work.

JPB, HG, DR, DC and KR have nothing to disclose.

**Data sharing**

De-identified individual participant data, together with a data dictionary defining each field in the set, will be made available to other researchers on request, subject to the 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 is 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 via a standard 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 release and requirements for data transfer, storage, archiving, publication and Intellectual Property. Requests are reviewed by the Trial Management Group (TMG) 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 TMG and approved by the Trial Steering Committee, as required.

Restrictions relating to patient confidentiality and consent will be limited 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 TMG and Trial Steering Committee, e.g. statistical analysis plan and informed consent form.

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**Tables and Figure Legends**

**Table 1 Clinical and pathological features of patients in the plasmaMATCH trial**

**Figures**

**Figure 1. Clinical ctDNA testing in advanced breast cancer**

Consort diagram of the plasmaMATCH study. Further detail on accuracy of ctDNA testing is provided in the supplementary material (appendix p17).

HR+ hormone receptor positive and HR- hormone receptor negative. HER2+ positive for *HER2* amplification or over-expression, HER2- negative. ER+ oestrogen receptor positive and ER- oestrogen receptor negative. TNBC – triple negative breast cancer. ABC – advanced breast cancer.

**Figure 2. Extended dose fulvestrant in *ESR1* mutant breast cancer (Cohort A)**

Waterfall plot of maximum change in tumour size in patients with *ESR1* mutations in ctDNA treated with extended-dose fulvestrant. Bars are colored by clonally dominant vs subclonal mutations. Boxes above indicate individual *ESR1* mutations, or polyclonal (multiple) *ESR1* mutations.

**Figure 3. Neratinib in *ERBB2* mutant breast cancer (Cohort B)**

Waterfall plot of maximum change in tumour size in patients with *ERBB2* mutations in ctDNA treated with neratinib with or without fulvestrant. Bars are colored by individual *ERBB2* mutations. Boxes above indicate tumour subtype, co-administration of fulvestrant, and receipt of prior fulvestrant therapy.

**Figure 4. Capivasertib in *AKT1* and *PTEN* mutant breast cancer (Cohorts C and D)**

A. Waterfall plot of maximum change in tumour size in patients, with HR+ cancer and with *AKT1* mutations in ctDNA, treated with capivasertib plus fulvestrant. Bars are colored by *AKT1* mutation type. Boxes above indicate prior fulvestrant therapy (Cohort C).

B. Waterfall plot of maximum change in tumour size in patients, with *AKT1* mutations in HR- breast cancer or in activating *PTEN* mutations, treated with capivasertib. Bars are colored by *AKT1* and *PTEN* mutation type. Boxes above indicate testing in ctDNA or tissue, and tumour subtype (Cohort D).

*PTEN* SNV – *PTEN* single nucleotide variant pathogenic mutation. *PTEN* truncating – *PTEN* truncating non-sense or frameshift mutation. *PTEN* deletion – *PTEN* homozygous deletion