Cell-free DNA Comparative Analysis of Genomic Landscape of First-line Hormone-receptor Positive Metastatic Breast Cancer From US and China

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Abstract

Background: The differential mutation landscape between ethnics is more comparable when using the identical detecting platform. We explored the differences in genomic landscape of patients with hormone-receptor positive (HR+), HER2-negative MBC of first recurrence or Stage IV at diagnosis from the United States (US) and China (CN).

Methods: Twenty-seven US patients and 65 CN patients had circulating tumor DNA (ctDNA) sequencing from plasma using the harmonized CLIA-certified, 152-gene PredicineCare™ liquid biopsy assay. Clinical outcomes were correlated with ctDNA variants. Progression-free survival (PFS) and overall survival analysis was performed for patients and compared using log-rank test.

Results: Mutations were detected in 23 of 27 (85%) US patients and 54 of 65 (83%) CN patients. The most common mutations detected included *TP53*, *PIK3CA*, *CDH1*, *AKT1*, *ESR1*, and *BRCA2* in both US and CN patients. *AKT1* (18.5% vs. 3.1%, $P = 0.008$) and *CDH1* mutations were more frequent in the US population (18.5% vs. 1.5%, $P = 0.021$), and gain of *FGFR1* was more common in the CN cohort (7.4% vs. 24.6%, $P = 0.048$). *PTEN* deletion (5.7 months vs. 13.2 months, $P = 0.03$) and *ESR1* alterations (9.0 months vs. 13.2 months, $P = 0.02$) were associated with significantly shorter PFS in CN cohort.

Conclusions: To our knowledge, this is the first real world study using a single harmonized ctDNA assay to profile plasma samples of patients from the US and CN. Differential prevalence of the mutations with therapeutic potential were found between US and CN.

Introduction

Metastatic breast cancer (MBC) is a heterogeneous disease with increased genomic complexity compared to primary breast cancer and is associated with known somatic mutations of variable biological value in different subtypes [1]. Hormone-receptor positive (HR+), HER2- breast cancer (BC) accounts for over 70% percent of breast cancer [2, 3]. Currently, endocrine therapy, CDK4/6 inhibitors, and PI3K/AKT/mTOR pathway inhibitors are being used in the clinic with several novel endocrine, targeted, and chemotherapy-based treatments in clinical development [4, 5].

Liquid biopsies, including cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), cell-free RNA, circulating tumor cells, exosomes, and protein have emerged as clinically relevant tools for prognosis, genomic characterization, and disease response in MBC. For MBC patients with metastasis in lung, liver, or bone, acquiring biopsies, particularly at serial timepoints, can be difficult and invasive [6]. Further, tissue biopsies may only reflect a spatially and temporally limited snapshot of disease biology [7]. In contrast, cfDNA assays have potential advantages with respect to tissue as non-invasive tools to reflect tumor heterogeneity that is feasible for facilitating serial disease monitoring. In multiple clinical trials for patients with MBC, ctDNA assays have been used for incorporating mutational profiling in drug development to identify potential predictive biomarkers [8]. For example, *ESR1* mutations were present in 37% of baseline samples and were enriched in patients with luminal A and *PIK3CA*-mutated tumors [9].

Another study using archived baseline plasma from SoFEA and PALOMA3 reported that *ESR1* mutations were present in 39.1% of patients in SoFEA and 25.3% in PALOMA-3. Analysis of cfDNA from the BOLERO-2 trial using droplet digital PCR (ddPCR) showed that 28.8% of patients had either D538G or Y537S mutations in *ESR1* [10] and 43.3% of the patients harbored H1047R, E545K, or E542K mutations in *PIK3CA* [11]. There was also a study using exome sequencing of plasma DNA from the PALOMA-3 study, which showed the dynamic changes in *PIK3CA* and *ESR1* mutations after treatment, demonstrating the utility of serial monitoring in MBC [12, 13].

Despite diagnostic and therapeutic advances for patients with MBC, breast cancer continues to have dramatic differences in terms of disease onset, management, and clinical outcomes between CN and western countries. For instance, age of breast cancer diagnosis is almost 10 years younger in Chinese patient populations as compared with patients in the United States (US) and the European Union (EU) [14]. Genomic profiling of breast cancer patients in the US and CN has reported controversial results. Studies across different tumor types report significant differences in certain gene pathways and molecular subtypes [15–19], while others showed similar genetic landscape [20, 21].

Few studies have explored the differences in genomic features of tumors, particularly based on ctDNA assessment, across populations. In this study, we used a harmonized ctDNA liquid biopsy test to systematically compare the genomic landscape of patients with HR + MBC at the time of first recurrence or *de-novo* metastatic diagnosis in the US and CN.

Materials And Methods

Patients

Two cohorts with HR+ and HER2- BC at the time of first relapse or *de-novo* metastatic pathological diagnosis from the US (February 2017 - October 2019) and CN (March 2018 - March 2019) participated in this study. The enrollment criteria were: 1). The patients had HR+ and HER2- relapse after surgery or *de-novo* MBC. HR+ was defined as $\geq 1\%$ for estrogen receptor (ER) and / or progesterone receptor (PR) positive; 2). Patients signed informed consent for additional blood to be collected for gene testing; 3). Age between 18 and 85 years; 4). At least one measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1; 5). Patients had a performance status score of Eastern Cooperative Oncology Group (ECOG) ≤ 1 ; 6). Patients had a life expectancy of at least 12 weeks, and adequate haematologic, hepatic, and renal function. Plasma samples were prospectively collected, 10 mL from each patient, before any treatment was initiated in the metastatic setting. Institutional Review Boards at each site approved the study in US (ethic No. NU16B06) and CN (ethic No. 2016KT75) .

Plasma cfDNA NGS testing

cfDNA testing was performed using the harmonized 152-gene PredicineCARE assay in two College of American Pathologist (CAP)-accredited laboratories separately, one was in the US (Predicine Inc.) and another one was located in CN (Huidu Shanghai Medical Sciences Ltd.).

Plasma cfDNA extraction

cfDNA was extracted using QIAamp circulating nucleic acid kit from plasma samples. Quantity and quality of the purified cfDNA were checked using Qubit fluorimeter and Bioanalyzer 2100.

Library preparation, capture and sequencing

5 to 20ng of extracted cfDNA was prepared for library construction including end-repair, dA-tailing, adapter ligation, and PCR amplification. The amplified DNA libraries with sufficient yields proceeded to hybrid capture. In brief, the library was hybridized overnight with the panel probes. Unbound fragments were then washed away. The purified libraries were QCed with Bioanalyzer 2100 and then paired-end 2x150bp sequenced using the Illumina sequencing platform.

Variant calling

Variants were called using a Predicine in-house developed analysis pipeline, starting from the raw sequencing data to the final mutation calls, which has been described in previous publications [22, 23]. Briefly, the pipeline first performed adapter trimming, barcode checking, and correction. Cleaned paired FASTQ files were outputted by the in-house pipeline and further aligned to the human reference genome build hg19 using BWA (version 0.7.15) alignment tool. Consensus bam files were then derived by merging paired-end reads originated from the same molecules (based on mapping location and unique molecular identifiers) as single strand fragments. Single strand fragments from the same double strand DNA molecules were further merged as double stranded. Both sequencing and PCR errors were deeply suppressed during this process. Candidate variants, consisting of point mutations, small insertions, and deletions, were identified across the targeted regions covered in the panel. Copy number variations were estimated at the gene level. The pipeline calculated the on-target unique fragment coverage, which was first corrected for GC bias, and was then adjusted to the probe level bias (estimated from a pooled reference).

Statistical analysis

Fisher's exact test was performed to compare the mutational prevalence between CN and US cohorts. The basic clinical characteristic comparison of the two cohorts were carried out using a t-test. Kaplan-Meier survival analysis was performed to analyze the correlation between genomic alterations and progression-free survival (PFS), and *p*-values were calculated using the log-rank test by comparing the patients with and without a particular genomic alteration. R (version 3.5) was used for statistic analysis. Survival and surminer R packages were used for survival analysis.

Results

Baseline patient characteristics

In total, there were 27 US patient at Northwestern University and 65 Chinese patients at Peking University Cancer Hospital enrolled in this study based on the inclusion criteria (see Materials and Methods). Median age of diagnosis was 51 years (range: 30–79) in the US cohort and 49 years (range: 27–82) years in the CN cohort. 87% of US patients and 82% of Chinese patients had received adjuvant therapy for primary BC, including chemotherapy and/or endocrine therapy. The sites of metastasis in decreasing order of frequency were liver, lung, and bone in the US cohort and lung, liver, and bone in the CN cohort. There were no significant differences found between US and Chinese samples with respect to age (median age was 51 in US and 49 years in CN), gender (all female) or primary tumor stages (mostly stage II or above in US and Chinese patients, 91.3% and 75.0%, respectively). Patients' clinical and pathological characteristics are summarized in Table 1.

Table 1
Comparison of baseline clinical characteristics between US and CN (n = 90)

Clinical characteristics	US (n = 27)	CN (n = 65)	<i>P</i>
Age at diagnosis			0.400
≤45 years	10 (37.0%)	21 (31.7%)	
>45 years	17 (63.0%)	44 (68.3%)	
HR intensity of primary tumor			0.005
≤25%	9 (33.3%)	7 (9.5%)	
>25%	18 (66.7%)	58 (90.5%)	
Tumor grade of primary tumor			0.306
I	2 (7.4%)	4 (6.2%)	
II	13 (48.1%)	32 (49.2%)	
III	8 (29.6%)	10 (15.4%)	
Unknown	4(14.8%)	19(29.2%)	
T stage of primary tumor			0.013
T1/2	15 (55.6%)	52 (80.0%)	
T3/4	10 (37.0%)	7 (10.8%)	
Unknown	2 (7.4%)	6 (9.2%)	
N stage of primary tumor			0.776
N0/1	14 (51.6%)	33 (50.8%)	
N2/3	12 (44.4%)	27 (41.5%)	
Unknown	1 (3.7%)	5 (7.7%)	
Previous neoadjuvant chemotherapy			0.022
No	11 (40.8%)	54 (83.1%)	
Yes	9 (33.3%)	11 (16.9%)	
Unknown	7 (25.9%)	0 (0.0%)	
Previous adjuvant chemotherapy			0.987
No	8 (29.6%)	24 (36.9%)	
Yes	15 (55.5%)	39 (60.0%)	

Clinical characteristics	US (n = 27)	CN (n = 65)	<i>P</i>
Unknown	4 (14.8%)	2 (3.1%)	
Previous adjuvant endocrine therapy			0.001
No	1 (3.7%)	20 (30.8%)	
SERM ^a	8 (29.6%)	33 (50.8%)	
AI ^b	8 (29.6%)	10 (15.4%)	
SERM + AI	5 (18.5%)	1 (1.5%)	
Unknown	5 (18.5%)	1 (1.5%)	
Disease free survival			0.119
≤6.0 months	9 (33.3%)	37 (56.9%)	
>6.0 months	10 (37.0%)	15 (23.1%)	
Unknown	8 (29.6%)	13 (20.0%)	
Liver metastasis			0.129
No	24 (88.9%)	50 (75.8%)	
Yes	3 (11.1%)	15 (24.2%)	
Lung metastasis			0.021
No	23 (85.2%)	40 (61.3%)	
Yes	4 (14.8%)	25 (38.7%)	
Brain metastasis			0.217
No	25 (92.6%)	64 (98.4%)	
Yes	2 (7.4%)	1 (1.6%)	
Bone metastasis			0.538
No	10 (37.0%)	25 (38.7%)	
Yes	17 (63.0%)	40 (61.3%)	
Lymph metastasis			0.078
No	16 (59.3%)	27 (40.3%)	
Yes	11 (40.7%)	38 (59.7%)	
Chest metastasis			0.156

Clinical characteristics	US (n = 27)	CN (n = 65)	<i>P</i>
No	27 (100.0%)	60 (91.9%)	
Yes	0 (0.0%)	5 (8.1%)	
Other metastasis			0.386
No	19 (70.4%)	48 (75.8%)	
Yes	8 (29.6%)	17 (24.2%)	
Previous neoadjuvant and/or adjuvant chemotherapy			0.017
No	0 (0.0%)	18 (27.7%)	
Yes	20 (74.1%)	45 (69.2%)	
Unknown	7(25.9%)	2 (3.1%)	
Liver and/or lung metastasis			0.021
No	20 (74.1%)	31 (48.4%)	
Yes	7 (25.9%)	34 (51.6%)	
First-line therapeutic regimen			0.000
Chemotherapy	0 (0.0%)	22 (33.8%)	
Hormonal therapy	3 (11.1%)	15 (23.1%)	
Chemotherapy followed by hormonal therapy	0 (0.0%)	28 (43.1%)	
Hormonal plus CDK4/6 inhibitor	23 (85.2%)	0 (0.0%)	
Unknown	1 (3.7%)	0 (0.0%)	
^a SERM: tamoxifen/toremifene			
^b AI: anastrozole/exemestane/letrozole			

Mutational and CNV landscape

The samples were tested by a harmonized NGS-based 152-gene PredicineCARE™ assay by Predicine Inc. in the US and by Huidu (Shanghai) in CN. SNV/Indel mutations were detected in 23 of 27 (85%) US patients and 54 of 65 (83%) Chinese patients. The number of mutations detected in each patient ranged from 0 to 9 in US patients and 0 to 9 in Chinese patients. CNVs, including both copy number gain and loss, were also detected in 13 of 27 (48%) US patients and 32 of 65 (51%) Chinese patients. The number

of CNVs detected in each patient ranged from 0 to 19 in US patients and 0 to 24 in Chinese patients. The top SNV/Indel mutations detected similarly included *TP53* (44.6%), *PIK3CA* (25.9%), *ESR1* (11.1%) and *BRCA2* (11.1%) in US patients, and *TP53* (33.8%), *PIK3CA* (44.6%), *ESR1* (12.3%) and *BRCA2* (9.2%) in Chinese patients. Alterations in *CDH1* (18.5% vs. 1.5%, $P = 0.008$) and *AKT1* (18.5% vs. 3.1%, $P = 0.021$) were significantly higher in the US cohort than in the Chinese cohort. The top CNV detected included copy number gain in the US vs. CN populations were the following: *CCND1* 11.1% vs 16.9, *CCND2* 14.8% vs 12.3, *CCND3* 14.8% 3.1%, and *MYC* 11.1% vs 13.8, respectively. There were no statistically significant differences in these genomic alterations. In contrast, *FGFR1* gain was significantly different between the US 7.4% and CN 24.6% ($P = 0.048$), while *CDK4* gain not significantly different between the US 7.4% and CN 1.5% populations ($P = 0.205$). For copy number loss, US vs CN were *ATM* loss 0 vs 16.9% ($P = 0.017$), *RB1* loss 3.7% vs 13.8% ($P = 0.145$), *BRCA2* 0 vs 7.7% ($P = 0.168$), *PTEN* 7.4% vs 4.6%, ($P = 0.460$), *BRCA1* 0 vs 1.5% ($P = 0.707$). (Figure 1A-C).

Survival analysis

The first-line treatment regimens for US and CN patients were different. The majority of patients (85.2%) in the US cohort were treated with standard of care hormonal therapy plus CDK4/6 inhibitor with a minority receiving single agent treatment, while no patients in the Chinese cohort received upfront CDK4/6 inhibitors. For further stratification, we divided the CN cohort into three subgroups, including those who received chemotherapy, hormonal therapy, and chemotherapy followed by hormonal therapy. For the US cohort, patients who received hormonal therapy plus CDK4/6 inhibitors were set up as group and their PFS was compared to the CN group. The result showed that US patients using hormonal plus CDK4/6 inhibitors had longer median PFS as compared to CN patients (26.9 months vs. 11.3 months, $P = 0.025$) (Figure 2A). We also carried out survival analysis for patients with any *AKT* activating or *PTEN* inactivating, or DDR deficiency mutations, in Chinese and US patients (Supplementary Figure 1, and data not shown). *PTEN* deletion was associated with shorter PFS of HR+ MBC patients in CN (5.7 months vs. 13.2 months, $P = 0.03$) (Figure 2B). This result was not encountered in US patients (5.4 months vs. 16.5 months, $P = 0.65$) (Figure 2B). Meanwhile, patients with cfDNA-based *ESR1* alterations had shorter PFS compared to the other patients in the first-line treatment after relapse in the CN patient cohort (9.0 months vs. 13.2 months, $P = 0.02$) (Figure 2C). These data was not statistically significant in US patients (11.2 months vs. 26.9 months, $P = 0.62$) (Figure 2C). Literature shows that cfDNA yield have been reported as a prognostic biomarker related to patient disease progression and prognosis [24]. However, there was no significant difference in cfDNA yield between Chinese and US patients from this study. (Supplementary Figure 2). In addition to mutational aspect, we also found that patients with liver or lung metastasis tend to have shorter PFS (14.3 months vs. 10.5 months, $P = 0.15$). This trend was also observed in CN cohort (14.3 months vs. 10.3 months, $P = 0.18$) but not in US cohort (13.5 months vs. 16.5 months, $P = 0.80$). (Data not showed)

Discussion

In this retrospective study, the gene mutation landscape in metastatic breast cancer was compared across two different populations. The results of the mutational analysis and prevalence varied for particular alterations between the two Ethnic groups. Currently, cfDNA-derived gene mutational assays have gained much attention in breast cancer (Fig. 3A and B). In prior work, the cfDNA-derived mutations were detected using next-generation sequencing (NGS) platforms in almost all of the studies. However, the means of extracting, processing and analyzing cfDNA samples were different, which can lead to a limited ability to compare differences across populations.

This retrospective, multicenter study is the first proof-of-concept study to report on the use of a single harmonized ctDNA assay in clinically uniform populations of HR + MBC from two institutions in the US and CN. The populations analyzed included newly recurred and *de-novo* MBC patients who had not been exposed to treatment in the advanced setting, but who had received adjuvant endocrine therapy in similar proportion following standard-of-care guidelines. The study demonstrates that cfDNA analysis can provide a reliable *real-world* assessment of the molecular landscape of luminal breast cancer and identify differences in genomic abnormalities between patients in the US and CN.

For cancer variants that were known to interfere with endocrine therapy combined with or without PI3K/AKT/mTOR or CDK4/6 inhibitors, such as PTEN gene deletion (i.e., copy number loss) or *ESR1* activating mutations and copy number gain were evaluated in the present study. Previous studies have shown that *PIK3CA* was mutated in 18–40% BC patients [25]. Some studies using cfDNA showed that *PIK3CA* mutations were present in 43.3% [12] and 25% [26] MBC patients in different studies. A recent study with cfDNA on a small cohort in CN observed that *PIK3CA* mutations were present in 29.4% (5/17) MBC patients [17]. In this study, we found significant difference of prevalence for *AKT1* (18.5% vs. 3.1%, $P = 0.008$) between US and CN patients, respectively. While the percentage was all within the previously reported ranges in other studies, the difference in *PIK3CA* detection in our study may have been secondary to differences in features of the study populations and raise the fascinating hypothesis of differential molecular pathways of endocrine resistance when comparing Ethnic groups treated with similar therapies.

In prior work, a mutational frequency of 0.4% was observed for *ATM* in 7,675 *BRCA1* & 2-negative breast cancer patients in a Chinese population [27, 28] and a mutational frequency range from 0.45 to 1.0% was found in the US and Europe [29, 30]. However, no available data regarding ctDNA based comparison between US and CN have been reported. Here, we found that *ATM* loss was more frequently observed in CN patients with HR+/HER2- mBC as compared to US (16.9% vs. 0.0%, $P = 0.017$). Previous studies showed that the mutational prevalence of *CDH1* detected using plasma of HR+/HER2- patients was 12.2% for US [31] and 5.0% for CN [21]. In contrast, our data showed that the *CDH1* mutation occurred more frequently in CN as compared to US (44.6% vs. 22.9%, $P = 0.021$). Our data also showed the CNV mutation prevalence of *FGFR1* gain was higher in CN patients when comparing to US (24.6% vs. 7.4%, $P = 0.048$). This result was in line with previous reports found in HR+/HER2- cohort of US (1.0 ~ 8.0%) and CN (13.0%) [12, 21, 31].

PTEN exerts its function in multiple ways including repressing tumor cell growth and cell survival. The nuclear *PTEN* exhibit phosphatase-independent tumor suppressive function such as regulation of chromosome stability, DNA repair, and apoptosis [32, 33]. *PTEN* loss has been identified in cfDNA of 25% mBC patients [26]. Here, we found that the difference of *PTEN* mutation frequency between the US and CN did not reach statistical significance (11.1% vs. 3.1%, $P = 0.15$). However, *PTEN* deletion, an agonist of PIK3CA/AKT pathway, was associated with shorter PFS of HR + MBC patients in CN ($P = 0.03$), but not in US, a finding that may have been limited by the smaller US sample size ($P = 0.65$).

On the other hand, circulating *ESR1* mutation is a poor prognostic factor in ER + MBC, Chandarlapaty et al reported that cfDNA *ESR1* mutations were associated with shorter OS of MBC patients from the BOLERO-2 study [10]. A recent meta-analysis by Zhang et al. demonstrated that plasma *ESR1* mutation carriers had significantly worse PFS compared to wild-type *ESR1* [34]. In the present study, the *ESR1* mutation prevalence between the US and CN was not significantly different. Patients with cfDNA-based *ESR1* copy number gain or mutation had shorter PFS compared to the other patients in the first-line treatment after relapse in the CN patient cohort ($P = 0.023$). Only two CN patients with *ESR1* were received letrozole. However, this finding was not observed in US patients ($P = 0.62$). The results mentioned above may partly due to the limited number of patients included in the US cohort. Future sequencing efforts and clinical trials should include patients of diverse ethnic backgrounds to explore the impact of differences in genomic landscape on probability of benefit from treatments. Interestingly, there is a trend that patients with liver or lung metastasis tend to have shorter PFS ($P = 0.18$) in CN cohort, which is not found in US cohort ($P = 0.80$). This may partly due to the massive application of CDK4/6 inhibitor in US patients, and this hypothesis need to be further demonstrated in larger cohort. Besides, we also noted that lung metastatic rate was higher in CN group as compared to US group ($P = 0.021$). previous study demonstrated that HR-positive patients with non-visceral metastases had a better prognosis than those with visceral metastases [35]. Thus we believe the higher lung metastatic rate of CN group may contribute to the shorter PFS.

Conclusion

Collectively, our data demonstrate differences in the ctDNA-based prevalence of certain genes between the US and CN and may suggest that baseline molecular characteristics. Meanwhile, as a *real-world* study, our data re-validated the advantage of using CDK4/6 inhibitor through comparison between US and CN population. It is also provoke us to discuss the possibility of popularize the combination of CDK4/6 inhibitor in HR-positive ABCs of CN, especially for whom with liver or lung metastasis. A larger validation cohort is required to confirm these findings.

Declarations

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Authors' contributions

Xiaoran Liu and Feng Xie did all the statistical analyses and constructed the tables and figures. Xiaoran Liu and Andrew A. Davis were also the main author of the manuscript. Huiping Li and Massimo Cristofanilli supervised the project. Huiping Li and Lorenzo Gerratana supervised the pathological evaluation. Huiping Li, Amir Behdad and Firas Wehbe evaluated the inclusion criteria of TMA and revised the manuscript. Xinyu Gui, Yifei Chen, Xiaoran Liu, Youbin Zhang and Qiang Zhang collected the raw clinical data. Huiping Li and Ami N. Shah provided the information on molecular subtypes and revised the manuscript. Yong Huang, Jianjun Yu, Pan Du and Shidong Jia provided the technology support of ctDNA sequencing and analyzing. All authors read and approved the final manuscript.

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Availability of supporting data

All data in the main text are publicly available after its publication. The supporting data of this study are available on request from the corresponding author (Huiping Li).

Ethics approval and consent to participate

The present study were approved by the medical ethics committee of Beijing cancer hospital (ethic No. 2016KT75) and of Lurie Comprehensive Cancer Center (ethic No. NU16B06). Written informed consent was obtained from all study participants at enrolled.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

ctDNA mutation landscapes of US and Chinese HR+ MBC patients. A, Mutation profile of US cohort. B, Mutation profile of Chinese cohort. C, Prevalence of gene mutation, copy number (CN) gain and CN loss in US and Chinese cohorts. P value was calculated using one-side Fisher's exact test.

Figure 2

Survival analysis of Chinese and US patients. A, Prolonged progression free survival (PFS) in US patients than in Chinese patients. All patients in US group received hormonal therapy combined with CDK4/6 inhibitor, whereas none of Chinese patients received CDK4/6 inhibitor. B, PFS of patients with or without PTEN detection. C, PFS of patients with or without ESR1 activating mutation or copy number gain. P value was calculated using log-rank test.

Figure 3

Schematic diagram of ctDNA detection. A, Detection of ctDNA from lung metastasis. B, Detection of ctDNA from liver metastasis.

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