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Dalpiciclib plus endocrine therapy in women with HR$^+$/HER2$^-$ advanced breast cancer and visceral crisis: a multicenter, nonrandomized, phase 2 DARVIN trial

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Visceral crisis in advanced breast cancer (ABC) represents a major challenge. Here we conducted a phase 2 study to evaluate dalpiciclib plus endocrine therapy (ET) in women with HR$^+$/HER2$^-$ ABC experiencing visceral crisis. The primary endpoint was the 6-month survival rate; secondary endpoints included overall survival (OS), progression-free survival (PFS), time to treatment failure (TTF), 3-month treatment failure rate (TFR), duration of disease control (DDC), objective response rate (ORR), disease control rate (DCR) and safety. Among 53 participants, 49 survived beyond 6 months, yielding a 6-month survival rate of 92.5% (95% confidence interval (CI): 81.8–97.9), meeting the primary endpoint. The ORR was 26.4%, DCR was 79.2% and 3-month TFR was 22.6%. The median PFS was 11.2 months (95% CI: 7.6–19.3), DDC was 14.1 months (95% CI: 8.4–21.5) and TTF was 10.2 months (95% CI: 6.4–15.6); the median OS was not reached. The most common grade ≥3 adverse events were neutrophil count decreased (77.4%) and white blood cell count decreased (54.7%). Exploratory analyses indicated that a baseline monocyte-derived cfDNA level below 0.0581 was associated with worse OS (hazard ratio: 4.79, 95% CI: 1.05–45.47, $P = 0.0394$), indicating a 'molecular crisis'. These findings support further evaluation of dalpiciclib plus ET in persons with ABC with visceral crisis (ClinicalTrials.gov: NCT05431504).

Visceral crisis affects approximately 60% of persons with advanced breast cancer (ABC), particularly in those with hormone receptor (HR)$^+$ and human epidermal growth factor receptor 2 (HER2)$^-$ disease[1–3]. Chemotherapy is the recommended treatment because of its capacity to induce rapid responses and higher response rates in persons with visceral crisis[4]. However, clinical guidelines do not define a preferred chemotherapy regimen and selecting the most appropriate regimen can be challenging because of frailty and underlying organ dysfunction[2]. This often results in the use of nonstandardized therapeutic approaches, including suboptimal dosing and frequent treatment interruptions. Additionally, the altered pharmacokinetics associated with liver dysfunction or ascites in these individuals can elevate the risk of treatment-related toxicities. Moreover, the exclusion of persons with visceral crisis from clinical trials further limits the availability of prospective data to inform evidence-based treatment decisions.

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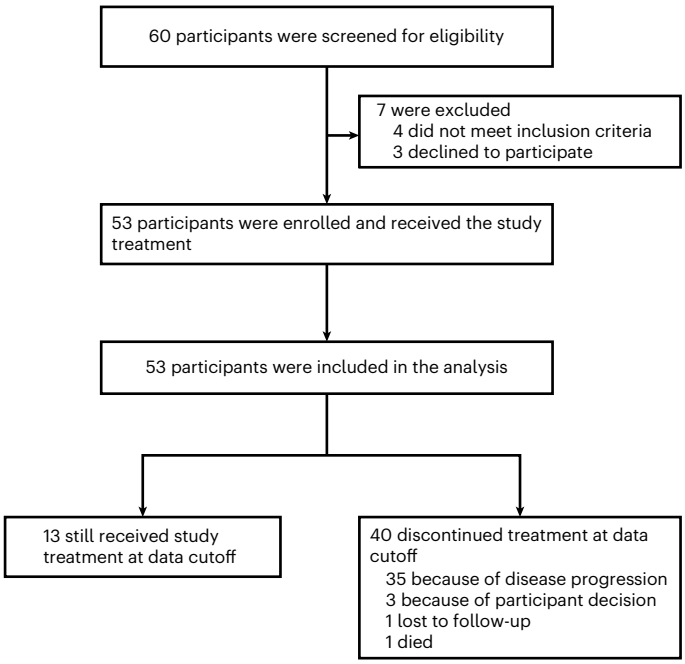

Fig. 1 | Study flowchart. Flow diagram showing patient enrollment, treatment administration, and analysis populations in the DARVIN study. A total of 53 patients were enrolled and received dalpiciclib plus endocrine therapy. All 53 patients were included in the full analysis set (FAS) and safety set (SS).

Inhibitors of cyclin-dependent kinases 4 and 6 (CDK4/6) in combination with endocrine therapy (ET) are now established as standard first-line treatment for persons with HR$^+$/HER2$^-$ ABC[5,6]. In women with clinically aggressive HR$^+$/HER2$^-$ ABC, the RIGHT Choice study showed that ribociclib combined with ET provided a notable progression-free survival (PFS) advantage and improved tolerability over chemotherapy of the investigator's choice[7]. However, subgroup analysis of participants experiencing visceral crisis (47.7%) revealed comparable PFS outcomes between ribociclib plus ET and chemotherapy (hazard ratio (HR) = 0.95)[8]. As such, the optimal treatment strategy for persons with ABC presenting with visceral crisis remains uncertain.

Dalpiciclib (Dal), an oral selective CDK4/6 inhibitor, has demonstrated significant efficacy in combination with ET in prior studies. The DAWNA-1 trial showed that Dal plus fulvestrant substantially prolonged PFS in participants with HR$^+$/HER2$^-$ ABC who had relapsed or progressed after prior ET[9]. The DAWNA-2 trial further corroborated these findings, revealing that first-line treatment with Dal plus letrozole or anastrozole significantly improved PFS compared to ET alone[10]. However, the efficacy of Dal plus ET (Dal + ET) in persons experiencing visceral crisis remains undefined. Therefore, this multicenter, phase 2 study aimed to evaluate the efficacy and safety of Dal + ET in participants with HR$^+$/HER2$^-$ ABC with visceral crisis.

Visceral crisis is defined in various ways, with most definitions remaining ambiguous. These typically encompass not only the presence of visceral metastases but also notable organ dysfunction, which leads to life-threatening conditions requiring immediate intervention[1]. Recent advancements in the Human Cell Methylation Atlas Project have established a robust foundation for tracing the origins of cell death and dynamically assessing organ damage through peripheral blood analysis[11,12]. In cardiovascular research, cell-free DNA (cfDNA) methylation markers have demonstrated strong correlations with disease severity and prognostic value for adverse survival outcomes[13,14]. Our prior studies also highlighted the potential of cfDNA methylation markers from target cells in critically ill persons with COVID-19, where they accurately predicted in-hospital mortality, outperforming conventional organ-level clinical indicators in prognostic accuracy[15]. Building on these findings, this study also aims to identify relevant cfDNA methylation markers for detecting ABC with visceral crisis.

Table 1 | Baseline characteristics of participants

Variables	Dal+ET (*n*=53)
Age (years), mean (s.d.)	55.8 (9.9)
ECOG performance status, *n* (%)	
0	24 (45.3)
1	23 (43.4)
≥2	6 (11.3)
PR positive, *n* (%)	44 (83.0)
ER positive, *n* (%)	52 (98.1)
Disease status, *n* (%)	
De novo	12 (22.6)
Relapse	41 (77.4)
Number of metastatic sites, *n* (%)	
<3	25 (47.2)
≥3	28 (52.8)
Metastatic sites, *n* (%)	
Bone	34 (64.2)
Brain	2 (3.8)
Liver	27 (50.9)
Lung	23 (43.4)
Lymph nodes	21 (39.6)
Prior lines of chemotherapy, *n* (%)	
0	34 (64.2)
1	14 (26.4)
≥2	5 (9.4)
Visceral crisis classification, *n* (%)	
Effusion	27 (50.9)
Symptomatic	9 (17.0)
Laboratory abnormalities	12 (22.6)
Others	5 (9.4)

Result

Baseline characteristics of participants

Between February 2023 and February 2024, 53 participants with visceral crisis were enrolled in the Dal + ET group. All participants were included in both the full analysis set (FAS) and safety set (SS). As of the data cutoff on July 31, 2025, the median follow-up was 19.4 months (interquartile range (IQR): 14.3–23.7). At the data cutoff date, 40 participants had discontinued the study, while 13 participants were still receiving treatment (Fig. 1). The mean age of participants was 55.8 ± 9.9 years, with the majority (88.7%) having an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Of the cohort, 22.6% had de novo ABC, while 77.4% had relapsed disease. Additionally, 64.2% of participants had not received prior chemotherapy for advanced disease. Visceral crisis presentations included effusion in 50.9% of participants, symptomatic crisis in 17.0%, laboratory abnormalities in 22.6% and 9.4% categorized under other types (Table 1).

Efficacy

In the FAS, 17 of the first 18 participants achieved a 6-month survival in the first stage, leading to the recruitment of an additional 35 participants for the second stage. Among the 53 participants, 49 survived

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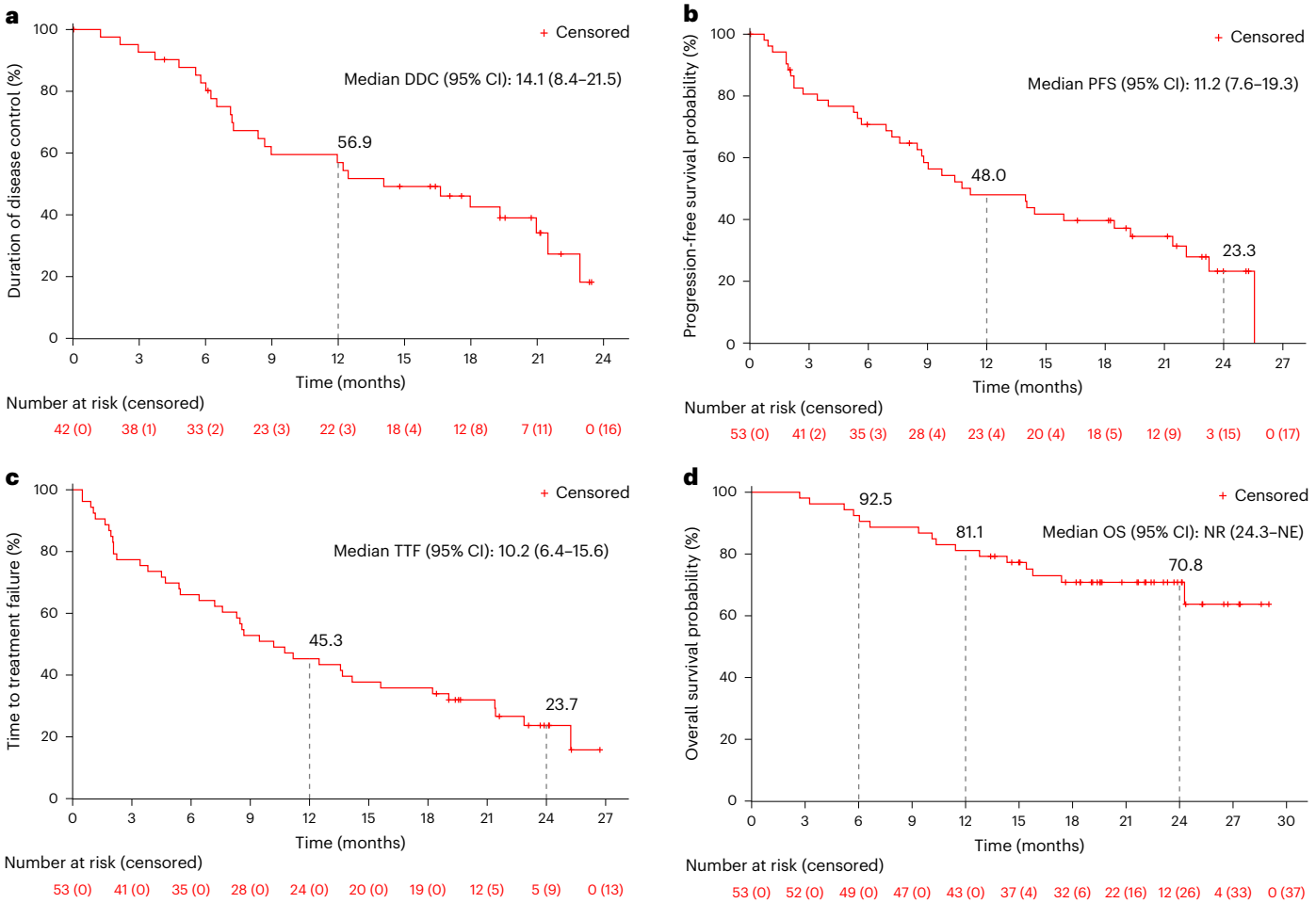

Fig. 2 | Kaplan–Meier survival curves for survival outcomes. a–d, DDC (**a**), PFS (**b**), TTF (**c**) and OS (**d**) in the FAS. NR, not reached.

beyond 6 months, yielding a 6-month survival rate of 92.5% (95% confidence interval (CI): 81.8–97.9), thereby rejecting the null hypothesis of a survival rate of 44% or lower. Using the Kaplan–Meier method, the estimated 6-month, 12-month and 24-month survival rates were 92.5% (95% CI: 81.1–97.1), 81.1% (95% CI: 67.8–89.4) and 70.8% (95% CI: 56.2–81.3), respectively. A total of 14 participants (26.4%) achieved a partial response and 28 participants (52.8%) had stable disease, resulting in an objective response rate (ORR) of 26.4% (95% CI: 15.3–40.3) and a disease control rate (DCR) of 79.2% (95% CI: 65.9–89.2). The 3-month treatment failure rate (TFR) was 22.6% (95% CI: 12.3–36.2). The median PFS was 11.2 months (95% CI: 7.6–19.3). Additionally, the median duration of disease control (DDC) was 14.1 months (95% CI, 8.4–21.5) and the median time to treatment failure (TTF) was 10.2 months (95% CI: 6.4–15.6) (Fig. 2).

One participant died before the first postbaseline efficacy assessment and was, therefore, excluded from the per-protocol set (PPS). Among 52 participants in the PPS analysis, 49 survived beyond 6 months, yielding a 6-month survival rate of 94.2% (95% CI: 84.1–98.8). Using the Kaplan–Meier method, the 6-month, 12-month and 24-month survival rates in the PPS were 94.2% (95% CI: 83.2–98.1), 82.7% (95% CI: 69.4–90.6) and 72.2% (95% CI: 57.5–82.5), respectively. The ORR was 26.9% (95% CI: 15.6–41.0) and the DCR was 80.8% (95% CI: 67.5–90.4). The 3-month TFR was 21.2% (95% CI: 11.1–34.7). The median PFS was 11.2 months (95% CI: 8.5–19.3). Additionally, the median DDC was 14.1 months (95% CI, 8.4–21.5) and the median TTF was 10.5 months (95% CI: 7.2–15.6) (Extended Data Fig. 1).

Among 27 participants presenting with effusion (50.9%), the 6-month, 12-month and 24-month survival rates were 96.8% (95% CI: 79.2–99.5), 87.1% (95% CI: 69.2–95.0) and 83.6% (95% CI: 65.0–92.8), respectively. The ORR was 32.3% (95% CI: 16.7–51.4) and the DCR was 90.3% (95% CI: 74.2–98.0). The 3-month TFR was 12.9% (95% CI: 5.0–30.8). The median PFS was 19.3 months (95% CI, 14.0–not estimable (NE)), the median DDC was 19.3 months (95% CI: 12.5–NE) and the median TTF was 18.2 months (95% CI: 9.5–22.9).

Efficacy between Dal + ET and chemotherapy groups before and after inverse probability of treatment weighting (IPTW)

This study used real-world data to construct an external control group, comprising participants with $HR^+/HER2^-$ ABC presenting with visceral crisis who had received chemotherapy. A total of 157 participants with $HR^+/HER2^-$ ABC and visceral crisis were included in the chemotherapy group, receiving chemotherapy between March 2016 and May 2024. Among them, 50.3% of participants presented with effusion, 3.2% with symptomatic crisis, 36.9% with laboratory abnormality-induced crisis and 9.6% with other types. After IPTW adjustment, the effective sample sizes were 53 for the Dal + ET group and 46.6 for the chemotherapy group, with baseline characteristics balanced across both groups (Supplementary Table 1). The Dal + ET group exhibited longer PFS compared to the chemotherapy group, with a median of 11.2 months versus 4.6 months, respectively (HR = 0.30, 95% CI: 0.18–0.50, $P < 0.0001$). Similarly, TTF was prolonged in the Dal + ET group, with a median of 10.2 months compared to 4.2 months in the chemotherapy group (HR = 0.31, 95% CI: 0.19–0.51, $P < 0.0001$). The median DDC was also longer in the Dal + ET group, at 14.1 months, compared to 5.1 months in the chemotherapy group (Extended Data Fig. 2).

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Table 2 | TEAEs occurring in ≥10% of participants in the Dal+ET group

Events, *n* (%)	Dal+ET (*n*=53)	
	Any grade	Grade ≥3
Any events	53 (100.0)	46 (86.8)
Hematologic events		
White blood cell count decreased	51 (96.2)	29 (54.7)
Neutrophil count decreased	51 (96.2)	41 (77.4)
Anemia	28 (52.8)	9 (17.0)
Platelet count decreased	21 (39.6)	1 (1.9)
Nonhematologic events		
Fatigue	19 (35.8)	0
AST increased	12 (22.6)	3 (5.7)
Rash maculopapular	10 (18.9)	2 (3.8)
Nausea	10 (18.9)	0
Pain	10 (18.9)	0
Hypoalbuminemia	9 (17.0)	1 (1.9)
ALT increased	8 (15.1)	1 (1.9)
Blood bilirubin increased	8 (15.1)	1 (1.9)
Blood creatinine increased	7 (13.2)	0
Alopecia	7 (13.2)	0
Hypocalcemia	6 (11.3)	0
Diarrhea	6 (11.3)	0
Hypertriglyceridemia	6 (11.3)	0
Peripheral edema	6 (11.3)	0
Dizziness	6 (11.3)	0

Safety

In the SS, the safety profile was consistent with previous reports. A total of 46 participants (86.8%) experienced treatment-emergent adverse events (TEAEs) of grade ≥3, with hematologic toxicities being the most prevalent. Grade ≥3 neutrophil count decreased occurred in 77.4% of participants, while grade ≥3 white blood cell count decreased was observed in 54.7%. Among nonhematologic grade ≥3 TRAEs, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increased were noted in 5.7% and 1.9% of participants, respectively (Table 2). Dose modifications because of TEAEs were required in 19 participants (35.8%). The most frequent causes were neutrophil count decreased (32.1%), white blood cell count decreased (24.5%) and anemia (13.2%). No participants discontinued treatment or died because of TEAEs.

cfDNA methylation analysis

A total of 221 plasma samples were analyzed for cfDNA methylation. Of these, 68 plasma samples were collected from participants with visceral crisis, including 36 baseline samples and 32 samples collected after one treatment cycle. Additionally, 104 blood samples were obtained from participants with breast cancer without visceral crisis in the validation cohort, with 72 of these samples coming from participants with visceral metastasis. To further validate the findings, we also analyzed 49 plasma samples from healthy individuals (Fig. 3a). Compared to healthy individuals, participants with breast cancer demonstrated significantly higher levels of cfDNA derived from breast cells ($P < 0.0001$) and monocytes ($P = 0.0007$) in their peripheral blood. Conversely, cfDNA derived from natural killer (NK) cells was lower in participants with breast cancer ($P < 0.0001$), while no significant difference was observed in cfDNA derived from T cells ($P = 0.8023$) between the two groups (Extended Data Fig. 3).

We then compared the cfDNA derived from various cell types at baseline across three participant groups: those with visceral crisis, those with visceral metastasis without visceral crisis and those with no visceral crisis or metastasis. The monocyte-derived cfDNA was significantly lower in the visceral crisis group compared to the participants without visceral crisis ($P < 0.0001$, $P = 0.0004$; Fig. 3b). No significant differences were observed in the NK cell-derived or T cell-derived cfDNA between participants with visceral crisis and those with visceral metastasis but no visceral crisis ($P = 0.7818$, $P = 0.9273$). Similarly, breast-cell-derived cfDNA did not differentiate between participants with visceral metastasis with or without visceral crisis ($P = 0.6947$).

Notably, in participants with visceral crisis, baseline monocyte-derived cfDNA was significantly correlated with overall survival (OS) (HR = 4.79, 95% CI: 1.05–45.47, $P = 0.0394$; Fig. 3c). Thus, monocyte-derived cfDNA below 0.0581 was identified as a 'molecular crisis' threshold to predict a poor prognosis. In the validation cohort, participants with no visceral crisis who exhibited molecular crisis also demonstrated significantly worse median OS (HR = 2.81, 95% CI: 1.52–5.19, $P = 0.0006$; Fig. 3d).

We further examined the level of cfDNA derived from different organ sources in predicting specific types of visceral crises. In participants with a bone-related crisis, osteoblast-derived cfDNA level was significantly higher compared to participants without crisis ($P = 0.0002$; Extended Data Fig. 4a). In participants with liver metastasis, the level of cfDNA derived from gallbladder cells was significantly elevated compared to those without liver metastasis ($P = 0.0066$). However, no significant differences were observed in the cfDNA derived from hepatocytes ($P = 0.6151$) or gallbladder cells ($P = 0.8101$) between participants with hepatic crisis and those with liver metastasis without crisis (Extended Data Fig. 4b). Notably, cortical neuron-derived cfDNA in participants with brain metastases was significantly higher than in participants without brain metastases ($P = 0.0234$; Extended Data Fig. 4c).

We also assessed dynamic changes of cfDNA derived from specific cells in peripheral blood of participants with visceral crisis after one cycle of treatment. After one cycle of Dal + ET treatment, the level of monocyte-derived cfDNA was significantly increased compared to baseline ($P = 0.0243$), whereas the level of breast-cell-derived cfDNA significantly decreased ($P = 0.0045$) (Extended Data Fig. 5a). Furthermore, in participants with a breast cell cfDNA level ≤ 0.0146 after one cycle of treatment, the median PFS was significantly longer than in those with a cfDNA level > 0.0146 (not reached versus 7.0 months, HR = 6.13, 95% CI: 2.09–17.99, $P = 0.0002$; Extended Data Fig. 5b). In the validation cohort, which consisted of participants without visceral crisis, breast-cell-derived cfDNA levels after one cycle of treatment also demonstrated a strong predictive value for PFS (HR = 3.39, 95% CI: 2.00–5.76, $P < 0.0001$; Extended Data Fig. 5c). Higher breast-cell-derived cfDNA levels (>0.0146) predicted a worse median PFS after treatment (1.6 months versus 4.3 months) in the validation cohort.

Discussion

The DARVIN study is, to our knowledge, the first to evaluate the efficacy and safety of Dal + ET in persons with visceral crisis, demonstrating a 6-month survival rate of 92.5% in the Dal + ET group, thereby meeting the primary endpoint. Importantly, Dal + ET was associated with a manageable safety profile, with no unexpected safety signals observed during the study.

CDK4/6 inhibition induces sustained cell-cycle arrest, enhances endocrine sensitivity and provides prolonged tumor control, whereas chemotherapy is associated with cumulative toxicity that can limit treatment continuity[16]. Thus, Dal + ET offers the dual advantages of rapid disease stabilization and durable efficacy with a more favorable tolerability profile, which is particularly relevant for persons in visceral crisis who require both immediate and sustained control. These mechanistic considerations are consistent with findings from recent trials comparing CDK4/6 inhibitor-based regimens with chemotherapy

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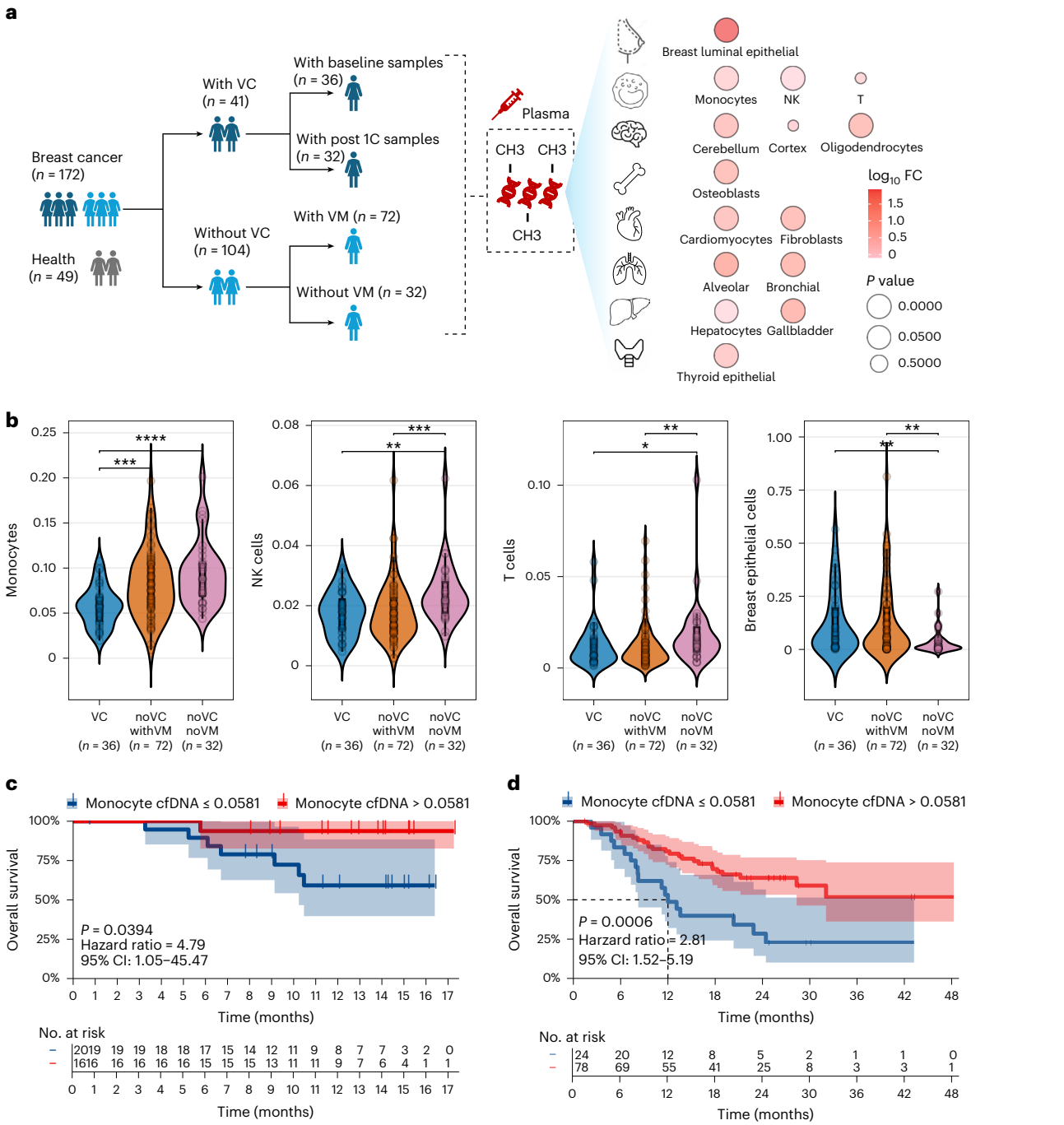

Fig. 3 | Plasma cfDNA methylation markers identify visceral crisis (VC) and stratify survival. a, Sample profile for biomarker analysis. cfDNA methylation testing was performed on plasma samples from 41 participants with breast cancer with VC and 104 participants with breast cancer without VC, together with 49 healthy individuals. Compared to healthy controls, several cfDNA methylation markers from different organ origins showed abnormalities in the plasma of participants with breast cancer. FC, fold change. **b**, Immune-cell-derived and breast-cell-derived cfDNA fractions in participants with VC ($n = 36$ plasma samples), visceral metastasis without crisis (noVCwithVM, $n = 72$ plasma samples) or no visceral metastasis (noVCnoVM, $n = 32$ plasma samples). Violin plots show the full distribution; each point is one plasma sample. Box plots show the median (center line), 25th and 75th percentiles (box bounds) and whiskers extending to the smallest and largest values within 1.5× the IQR. Global three-group comparisons used two-sided Kruskal–Wallis tests (monocytes, $P < 0.0001$; NK cells, $P = 0.0015$; T cells, $P = 0.0294$; breast epithelial cells, $P = 0.0054$). Brackets denote exploratory pairwise two-sided Wilcoxon rank-sum tests between subgroups; P values were not adjusted for multiple comparisons. Exact P values were reported where calculable and normal approximation was used when ties precluded exact calculation. Pairwise P values in the order noVCwithVM versus VC, noVCnoVM versus VC and noVCnoVM versus noVCwithVM were as follows: monocytes, $P = 0.0004$, $P < 0.0001$ and $P = 0.1587$; NK cells, $P = 0.7818$, $P = 0.0037$ and $P = 0.0006$; T cells, $P = 0.9273$, $P = 0.0402$ and $P = 0.0098$; breast epithelial cells, $P = 0.6947$, $P = 0.0024$ and $P = 0.0044$. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$. **c**,**d**, Kaplan–Meier estimates of OS by plasma monocyte-derived cfDNA fraction in participants with VC (**c**; $n = 36$ participants) or without VC (**d**; $n = 102$ participants). Shaded bands show 95% CIs and risk tables show numbers at risk. In **c**, $n = 16$ participants had >0.0581 (one event) and $n = 20$ had ≤0.0581 (seven events). The HR for ≤0.0581 versus >0.0581 was 4.79 (95% CI: 1.05–45.47), estimated by Firth-penalized Cox regression because of sparse events, with two-sided log-rank $P = 0.0394$. In **d**, $n = 78$ participants had >0.0581 (26 events) and $n = 24$ had ≤0.0581 (17 events). The HR for ≤0.0581 versus >0.0581 was 2.81 (95% CI: 1.52–5.19), estimated by Cox proportional hazards model, with two-sided log-rank $P = 0.0006$. Post 1C, after one cycle of treatment.

in HR$^+$/HER2$^-$ ABC. The PEARL trial showed that palbociclib plus ET achieved efficacy comparable to capecitabine with improved safety profiles and quality of life[17], while the KCSG-BR15-10 trial demonstrated significantly longer PFS with palbociclib plus ET than with capecitabine in premenopausal women[18]. Most notably, the RIGHT Choice trial established that ribociclib plus ET provided superior PFS and better tolerability compared to combination chemotherapy in the premenopausal population with aggressive disease[7]. Our study extends these findings by focusing exclusively on persons with visceral crisis and demonstrates that Dal + ET can achieve both rapid and durable disease control with manageable toxicity. To verify the reliability of these findings, validation in a large-scale clinical trial will be essential.

The RIGHT Choice study evaluated the efficacy of ribociclib combined with ET versus chemotherapy in participants with clinically aggressive HR$^+$/HER2$^-$ ABC. This study demonstrated a significant PFS advantage for ribociclib plus ET over chemotherapy[7]. Of the participants enrolled, 106 (47.7%) were assessed by investigators as having visceral crisis at the study's initiation, primarily according to the ABC 3 guidelines[19]. However, subgroup analysis revealed that the PFS benefit was less pronounced in participants with visceral crisis (HR = 0.95)[8], highlighting the need for more refined criteria to identify those who would benefit most from CDK4/6 inhibitors in combination with ET. In contrast, our study used the updated ABC5 guidelines[2], with all participants meeting the criteria for visceral crisis. Moreover, our cohort differed notably from the RIGHT Choice trial. While 62.5% of participants in the ribociclib plus ET arm of the RIGHT Choice study had newly diagnosed ABC and no prior systemic treatment[7], only 22.6% of our participants presented with de novo ABC and 35.8% had received prior chemotherapy in the advanced setting. Although neither study reached maturity in OS data, our findings further support the potential of CDK4/6 inhibitors in combination with ET as a promising treatment option for persons with HR$^+$/HER2$^-$ ABC with visceral crisis. Notably, approximately half of the participants in our trial met the criteria for visceral crisis on the basis of pleural or peritoneal effusion alone. Consistent with this, participants presenting with effusion demonstrated more favorable outcomes than the overall study population, as reflected by higher survival rates and longer PFS. This heterogeneity suggests that effusion-dominant presentations may represent a distinct clinical subset within visceral crisis. Additional studies are needed to identify the subgroups most likely to benefit from CDK4/6 inhibitors in this high-risk population[16].

In the DARVIN study, Dal + ET demonstrated a manageable safety profile, with no unexpected safety signals observed throughout the treatment period. The majority of grade ≥3 TRAEs were hematologic, primarily involving decreases in neutrophil and white blood cell counts. Nonhematologic grade ≥3 or higher TEAEs, such as elevations in AST and ALT, were relatively infrequent. This safety profile aligns with findings from the RIGHT Choice study, which reported that ribociclib plus ET was associated with a lower incidence of symptomatic AEs compared to chemotherapy, as well as a reduced rate of TRAEs leading to treatment discontinuation[7].

The definition of visceral crisis varies widely across studies, with criteria often depending on clinical context and investigator judgment. As a liquid biopsy technique with high sensitivity and specificity, cfDNA methylation has been used for the early detection of various malignancies[20–22]. To our knowledge, this is the first study to define a molecular crisis on the basis of cell-specific cfDNA methylation signatures. Molecular crisis, characterized by low levels of monocyte-derived cfDNA methylation, was associated with worse prognosis among participants with visceral crisis and may help to better identify those who are truly in a high-risk crisis state, beyond clinical features alone. We further validated this finding in an independent cohort, where participants with low baseline monocyte-derived cfDNA levels had poor survival prognosis, even in the absence of visceral crisis. The level of breast-cell-derived cfDNA correlates with the disease burden of breast cancer to some extent. Furthermore, changes in the level of breast cell cfDNA after one cycle of treatment can predict PFS in both cohorts. However, tumor burden alone does not necessarily correlate with the individual's crisis state and poor prognosis. Our findings suggest that high-resolution cfDNA methylation testing, particularly assessing systemic immune status through peripheral blood mononuclear cell levels, may more accurately identify persons truly in crisis among those with visceral metastases. The use of cfDNA methylation testing to identify persons with molecular crisis, who have a poor prognosis, and to predict the efficacy of antitumor therapies warrants further validation in larger, more diverse cohorts.

Previous cardiovascular studies have identified that myocardial cell-derived cfDNA level is significantly elevated in persons with acute myocardial infarction, highlighting its potential for early detection of myocardial injury[13]. In pulmonary hypertension, cfDNA derived from myocardial cells and the vascular endothelium has been strongly associated with disease severity and prognosis, including adverse survival outcomes[14]. This study also examined the crisis status of specific organs. The levels of cfDNA from organs such as bone, liver and brain were closely associated with breast cancer metastasis to these locations. Elevated cfDNA levels in osteoblasts were indicative of bone-related crises. However, cfDNA levels derived from hepatocytes and gallbladder cells did not correlate with liver crises in participants with liver metastasis. This may be because of the limitations of the liver-related markers in our study, which lack representation of bile duct cells, hepatic stellate cells and hepatic endothelial cells, thus limiting the ability to fully assess liver crisis. Expanding the current cfDNA methylation marker panel to enhance the sensitivity in assessing liver crisis is necessary.

This study had several limitations. First, conducting randomized controlled trials in persons with visceral crisis poses inherent challenges, largely because of ethical considerations, individual preferences and the scarcity of feasible treatment options. Accordingly, this study was designed as a nonrandomized, phase 2 trial and the findings should be interpreted with caution. Second, given the high-risk nature of visceral crisis, OS is a more clinically relevant endpoint than PFS for this population. Therefore, we selected the 6-month survival rate as the primary endpoint of this study. However, the limited follow-up duration precludes definitive conclusions regarding the long-term survival benefit of Dal + ET. A further limitation of this study is the inclusion of an external control cohort, which, although prespecified in the study design, is inherently subject to potential bias and confounding; therefore, the results of these exploratory comparisons should be interpreted with caution. Furthermore, this study explores cfDNA methylation as a prognostic and predictive biomarker in persons with ABC experiencing visceral crisis. While our results indicate high potential for this approach, they should be considered hypothesis generating because of the modest sample size and the single-arm study design. Further validation in larger, systematically designed prospective studies will be essential to confirm the correlation among cfDNA methylation patterns, treatment response and long-term outcomes.

In conclusion, the DARVIN study met its primary endpoint, demonstrating a 6-month survival rate of 92.5% in the Dal + ET group. Moreover, Dal + ET exhibited a manageable safety profile, with no unexpected safety signals observed throughout the study. These findings suggest that Dal + ET could be a therapeutic option for persons with HR$^+$/HER2$^-$ ABC experiencing visceral crisis. Furthermore, molecular crisis, as defined by monocyte-derived cfDNA, may identify individuals truly in crisis. The predictive value of methylation-based cfDNA biomarkers for visceral crisis warrants further validation in clinical practice.

Methods

Study design and participants

This multicenter, phase 2 trial was conducted across seven centers in China, with ethical approval granted by the Ethics Committee of the

Cancer Hospital, Chinese Academy of Medical Sciences (approval no. 22/106–3307), as well as all participating centers. The study was registered before participant enrollment (NCT05431504) and written informed consent was obtained from all participants. This study was reported in accordance with the CONSORT guidelines[23].

Eligible participants were postmenopausal and premenopausal or perimenopausal women aged ≥18 years, with histologically or cytologically confirmed ABC positive for progesterone or estrogen (ER^+ or PR^+) receptors and negative for HER2. Participants with measurable disease, as defined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, were included. Candidates for curative surgical resection or radiotherapy were excluded. Participants had to be deemed unsuitable for chemotherapy because of unwillingness, inability to tolerate chemotherapy or investigator assessment of contraindications. Visceral crisis was defined as the presence of symptoms attributable to symptomatic brain or visceral metastases, with at least one of the following criteria: carcinomatous meningitis or leptomeningeal metastasis, pleural effusion, ascites, abdominal pain because of liver or peritoneal metastasis, dyspnea resulting from pleural effusion, liver enzyme levels > 2× the upper limit of normal (ULN), bilirubin levels > 1.5× the ULN (excluding Gilbert's syndrome or biliary obstruction), pathologically confirmed bone marrow metastasis or hemoglobin levels < 100 g L^{-1}. Exclusion criteria included primary resistance to ET (defined as disease recurrence within 2 years of adjuvant ET or progression within 6 months of first-line ET in advanced disease), receipt of more than three prior lines of ET, prior treatment with any CDK4/6 inhibitor and bone-only metastasis. Full inclusion and exclusion criteria are detailed in the study protocol.

Procedures

Eligible participants were administered oral Dal (150 mg once daily, on a dosing schedule of 3 weeks on, 1 week off) in combination with ET of the investigator's choice. Tumor response was evaluated according to RECIST 1.1, using computed tomography or magnetic resonance imaging scans at baseline, after every two cycles for the first six cycles and every three cycles thereafter until disease progression, death, withdrawal of consent, initiation of new anticancer treatment or study completion, whichever occurred first. Adverse events were graded by investigators according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0).

Management of hematologic toxicities was prespecified in the protocol. For neutrophil count decreased and white blood cell count decreased, grade 1–2 events were managed with observation and monitoring alone, grade 3 events required temporary treatment interruption until recovery to grade ≤2, with granulocyte colony-stimulating factor permitted at the investigator's discretion, and grade 4 events or recurrent grade 3 events required treatment interruption until recovery, followed by resumption at a reduced dose. For anemia, grade 1–2 events were managed with observation and supportive care, grade 3 events required treatment interruption until recovery to grade ≥2, with red blood cell transfusion or erythropoietin as needed, and grade 4 events required interruption and transfusion, with dose resumption at a reduced level after recovery. For platelet count decreased, grade 1–2 events were managed with observation, grade 3 events required dose interruption until platelet counts recovered to $\geq 75 \times 10^9$ per L, with platelet transfusion administered as clinically indicated, and grade 4 events required treatment interruption and transfusion, followed by dose resumption at a reduced level after recovery.

Endpoints

The primary endpoint was the 6-month survival rate, defined as the proportion of participants alive at 6 months following treatment initiation. Secondary endpoints included OS, PFS, TTF, 3-month TFR, DDC, ORR, DCR and safety. Detailed definitions of these endpoints are provided in the study protocol.

External control chemotherapy group

This study used real-world data to construct an external control group, comprising participants with HR^+/$HER2^-$ ABC presenting with visceral crisis who had received chemotherapy. The chemotherapy group included participants treated either at an earlier time (from January 2018 onwards) or during the same period as the Dal + ET group. Inclusion and exclusion criteria for the chemotherapy group were broadly aligned with those of the Dal + ET group, differing only in treatment approach. Full details are provided in the study protocol. Participants in the chemotherapy group received chemotherapy as prescribed by their treating physician, with dosages determined according to the approved labeling for each agent. Treatment continued until disease progression, unacceptable toxicity, participant-initiated discontinuation or the onset of other medical conditions deemed by the physician to be incompatible with ongoing therapy. Tumor imaging evaluations and other clinical assessments for the chemotherapy group were performed in accordance with standard clinical practice.

Sample collection and DNA extraction

Plasma samples from participants with visceral crisis in this clinical trial were collected at baseline (within 1 week before treatment initiation) and after one cycle of treatment (at 4 weeks ± 3 days). For further validation, an independent cohort of participants with breast cancer without visceral crisis was used for cfDNA methylation analysis. This cohort was part of a phase 2 clinical trial (NCT04389073) designed to assess the safety and efficacy of metronomic vinorelbine combined with a PD1 antibody[24]. The inclusion criteria were as follows: participants aged >18 years, histologically confirmed $HER2^-$ metastatic breast cancer and a history of no more than one prior line of chemotherapy. Peripheral blood mononuclear cell samples from these participants were collected at baseline (within 1 week before treatment initiation) and after one cycle of treatment. Plasma cfDNA was extracted using the MagMAX cfDNA isolation kit (Thermo Fisher) and the TANBead Maelstrom 2400 extraction instrument in accordance with the manufacturer's instructions. DNA quantification and quality control were performed using the Qubit dsDNA HS assay kit (Thermo Fisher) and the Qsep100 automated Bio-Fragment Analyzer (Bioptic).

Targeted methylation sequencing

A 1% mass ratio of negative and positive controls was incorporated into the cfDNA before bisulfite conversion. The bisulfite-converted product was then purified and eluted using the EpiArt Magnetic DNA methylation bisulfite kit, following the manufacturer's protocol. During single-strand library construction, DNA pretreatment was performed on the bisulfite-converted product by incubating it at 95 °C for 3 min in a PCR instrument, followed by cooling on ice for 2 min. Subsequent steps, including 3′-adaptor ligation, complementary strand extension, postextension product purification, 5′-adaptor ligation and amplification, were carried out sequentially using the Hieff NGS Methyl-seq DNA library prep kit (Yeason). The concentration and fragment size of the library were assessed using the Qubit dsDNA HS assay kit (Thermo Fisher) and the Qsep100 automated Bio-Fragment Analyzer (Bioptic).

Hybridization and capture of the bisulfite library were performed using the self-developed T36 hybridization kit (Geneplus-Suzhou). During the process, the bisulfite library underwent preblocking, hybridization with a customized targeted methylation panel, elution, amplification and purification. Quality control of the library was performed using the Qubit dsDNA HS assay kit (Thermo Fisher) and the Qsep100 automated Bio-Fragment Analyzer (Bioptic). Sequencing was conducted in paired-end 150-bp mode on the DNBSEQ-T7RS high-throughput sequencing platform (MGI), achieving a mean depth of 951.66× (range: 242.52×–1742.68×) with nonoverlapping reads within a template.

The raw sequencing data underwent quality control, genomic alignment, methylation detection and quantitative assessment of organ injury, as outlined below. First, the raw data were filtered to remove

adaptor sequences, low-quality reads and poly(C) sequences introduced by the bisulfite single-stranded library using fastp software (version 0.23.4). The clean reads were aligned to the reference genome (hg19) and sorted, while repetitive sequences were removed using Bismark software (version 0.23.0). SAMtools (version 1.9) was used to generate the resulting BAM file. Quality control of the BAM file was performed with NCbamInfo (version 1.9.1) and GCTK (version 7.0.1). The libraries exhibited a high mapping rate of 85.86%, with a range of 80.51–89.72%.

Using wgbstools (version 0.2.0), the BAM files were converted into pat.gz files containing fragment alignment positions and methylation statuses. The number of UXM fragments containing ≥3 CpG sites in the marker regions was then extracted using Homog as input for the quantification algorithm. The cell fraction was quantified using the maximum likelihood estimation (MLE) algorithm, which determined the most probable fraction of target cells through grid search on the basis of the counts of fully unmethylated fragments (≥3 CpG sites) and the total number of fragments with ≥3 CpG sites in the marker region. Additionally, background noise from highly abundant nontarget cells in plasma was subtracted during the MLE calculation.

Cell-type-specific methylation markers

This study used human methylation data sourced from previous literature[11]. Cell-type-specific methylation markers traceable in cfDNA were selected to reveal cell-specific tissue injury and cfDNA origins, in accordance with the criteria outlined in earlier studies[15]. A total of 19, 19, 23, 16, 90, 12, 48, 144, 76, 34, 45, 34 and 19 methylation blocks were used as markers for breast luminal epithelial cells, immune cells (NK cells, T cells and monocytes), cortical neurons, osteoblasts, cardiac cells (fibroblasts and cardiomyocytes), lung cells (alveolar and bronchial epithelial cells), hepatocytes, gallbladder cells and thyroid epithelial cells, respectively.

Statistical analysis

This study used a Simon two-stage design with a one-sided α of 0.1, targeting a statistical power of at least 80%. The null hypothesis posited a 6-month survival rate of 44% (ref. 3), while the alternative hypothesis anticipated a 6-month survival rate of 60% in the Dal + ET group[25]. In the first stage, an initial cohort of 18 evaluable participants was enrolled. If nine or more participants were alive at 6 months, the study proceeded to the second stage, enrolling an additional 35 participants, for a total of 53 evaluable participants. If more than 27 participants survived at 6 months in the full cohort, the treatment regimen was considered promising and suitable for further investigation.

Efficacy analyses were primarily conducted in the FAS, which included all enrolled participants who received at least one dose of study treatment. The PPS comprised all participants in the FAS who did not experience major protocol deviations that could potentially affect efficacy outcomes. Safety analyses were performed in the SS, which included all participants who received at least one dose of study treatment. The 6-month survival rate, defined as the primary endpoint, was analyzed as a binary outcome and estimated with a 95% CI using the Clopper–Pearson method. Secondary time-to-event endpoints, including OS, PFS and TTF, were analyzed using the Kaplan–Meier method. Median survival times were estimated using Kaplan–Meier curves, with 95% CIs calculated according to the Brookmeyer–Crowley method. For other categorical endpoints, participant counts and percentages were summarized and corresponding 95% CIs were computed using the Clopper–Pearson method.

To ensure comparability between the Dal + ET and chemotherapy groups, IPTW was applied to adjust for key baseline covariates, including age, ECOG performance status (0 or 1 versus ≥2), disease status (de novo versus relapse), number of metastatic sites (<3 versus ≥3), prior treatment lines (0, 1 or ≥2) and type of visceral crisis (effusion, symptomatic, laboratory abnormalities or others). We applied IPTW methods to estimate the average treatment effect on the treated. Standardized mean differences were calculated after adjustment to assess baseline balance between the groups. Efficacy endpoints were compared across groups, with time-to-event outcomes analyzed using the log-rank test. HRs and 95% CIs were estimated using a Cox proportional hazards model. All statistical analyses were performed using SAS software (version 9.4 or later).

For biomarker analysis, the normality of continuous variables was assessed per group using the Shapiro–Wilk test and homogeneity of variances was assessed using Levene's test (rstatix version 0.7.2). As all cfDNA fraction measurements were nonnormally distributed, between-group comparisons were performed using two-sided Wilcoxon rank-sum tests (two groups) or Kruskal–Wallis tests (three or more groups). Exploratory pairwise comparisons are reported without multiplicity adjustment. For survival analyses, between-group differences were evaluated using a two-sided log-rank test and HRs with 95% CIs were estimated using Cox proportional hazards regression (survival version 3.8–3, survminer version 0.5.0). The proportional hazards assumption was verified using Schoenfeld residuals. For one survival comparison in which a group contained only a single event, Firth-penalized Cox regression was used (coxphf version 1.13.4). Optimal cutpoints for cell fractions were identified using maximally selected rank statistics (maxstat version 0.7-26). All analyses were performed in R version 4.5.1. A P value < 0.05 was considered statistically significant.

Reporting summary

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

Data availability

The raw sequence data of cfDNA methylation reported in this paper were deposited to the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences, under accession number HRA011705. Deidentified individual participant data that underlie the results reported in this article (including baseline characteristics, efficacy outcomes and safety data), along with aggregated cfDNA methylation profiles, will be made available to qualified researchers upon reasonable request. Requests for access should be directed to the corresponding author, F.M. (email: drmafei@126.com). Data will be shared for noncommercial academic research purposes only and will require a data sharing agreement with the sponsoring institution. The study protocol and statistical analysis plan are provided alongside this publication. Source data are provided with this paper.

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Author contributions

H.M., Y.T., L.C., H.L., X.W., J.Y., Y.W., Q.S., Y.H., J.L., Z.P. and F.M. participated in the design, execution and interpretation of the reported experiments and results. H.M., X.Y., R.C., D.L., X.P., S.W. and F.M. contributed to sample acquisition and data analysis. H.M., Q.S, Y.H. and T.F. performed statistical analysis. H.M., S.W., R.C. and X.Y. performed biomarker analysis. H.M. and F.M. drafted the manuscript, with all authors providing input, revisions and feedback. F.M. supervised all aspects of the research.

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Competing interests

S.W., R.C. and X.Y. are employees of Beijing Geneplus Technology. J.L., Z.P. and T.F. are employees of Jiangsu Hengrui Pharmaceuticals. The other authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s43018-026-01208-0.

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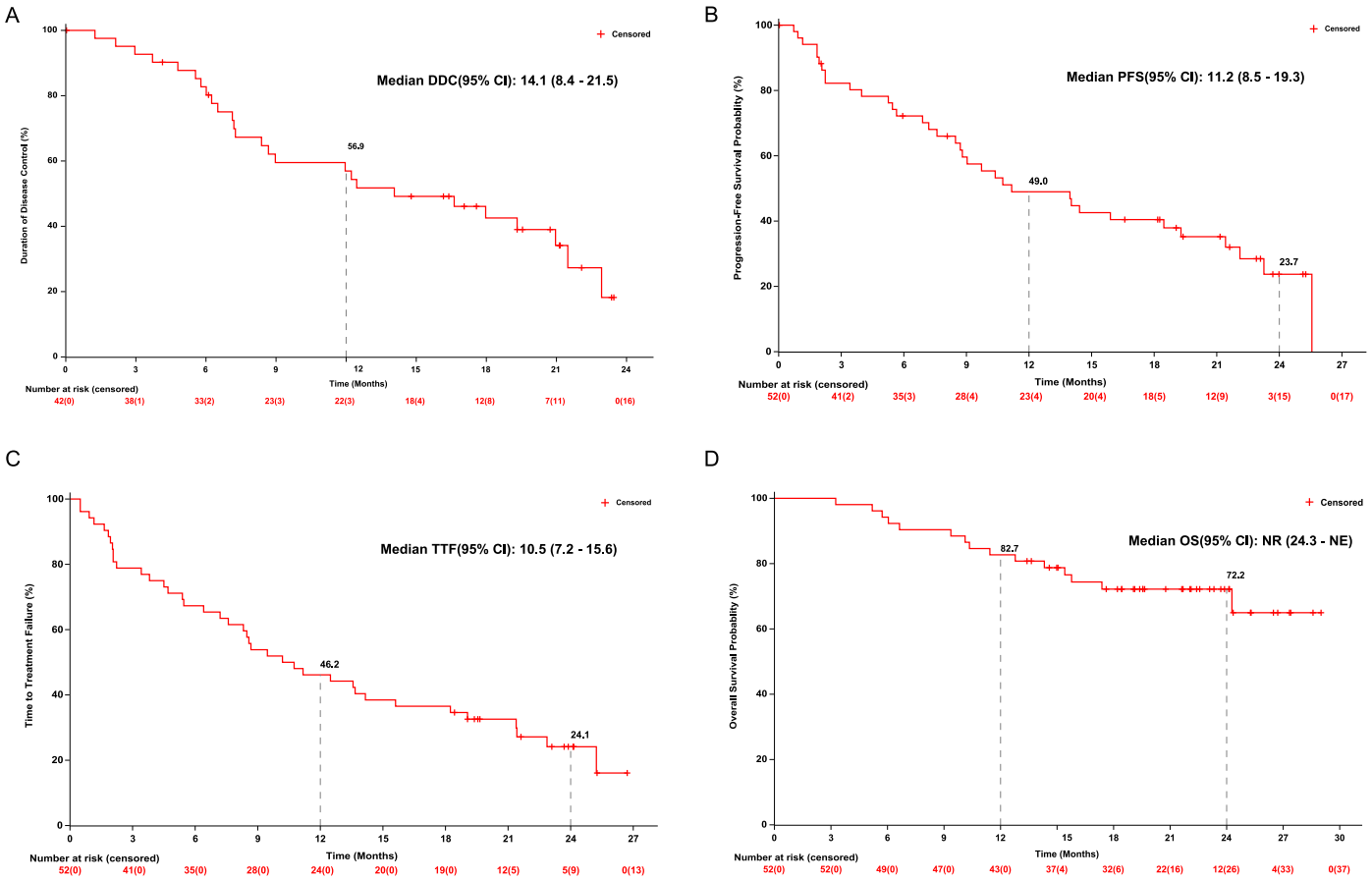

Extended Data Fig. 1 | Kaplan-Meier survival curves for survival outcomes in the per protocol set. (**a**) duration of disease control (DDC), (**b**) progression-free survival (PFS), (**c**) time to treatment failure (TTF), and (**d**) overall survival (OS). Dal+ET, dalpiciclib plus endocrine therapy; CI, confidence interval; NE, not estimated; NR, not reached.

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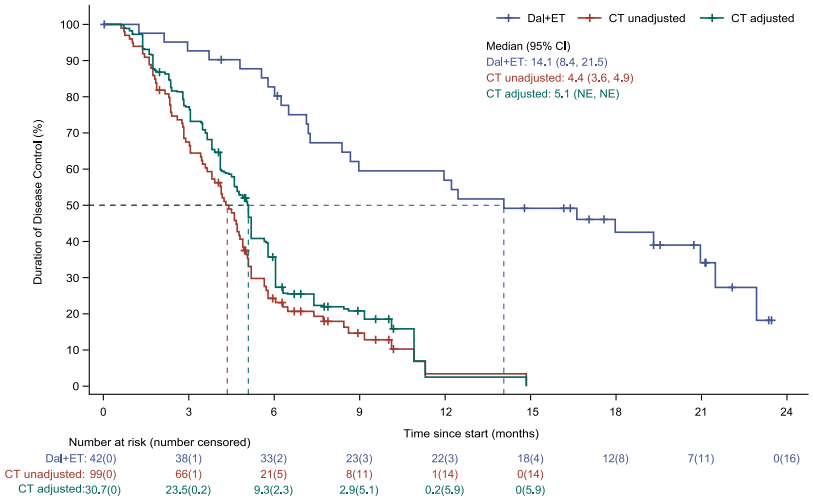

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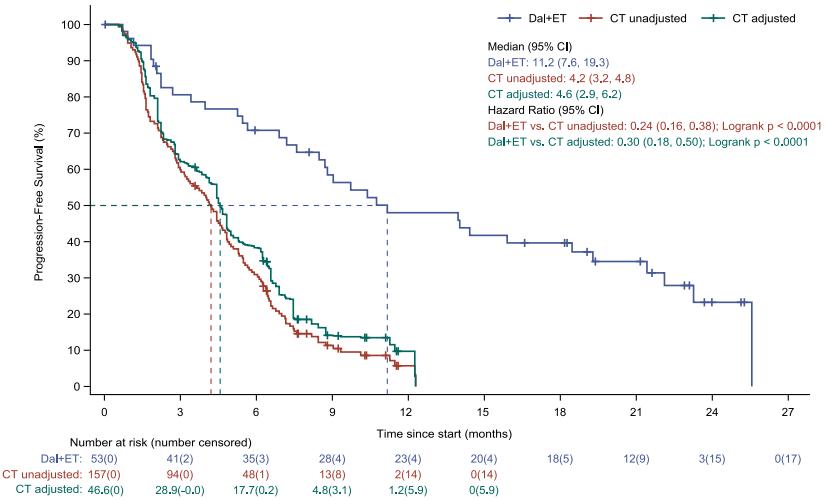

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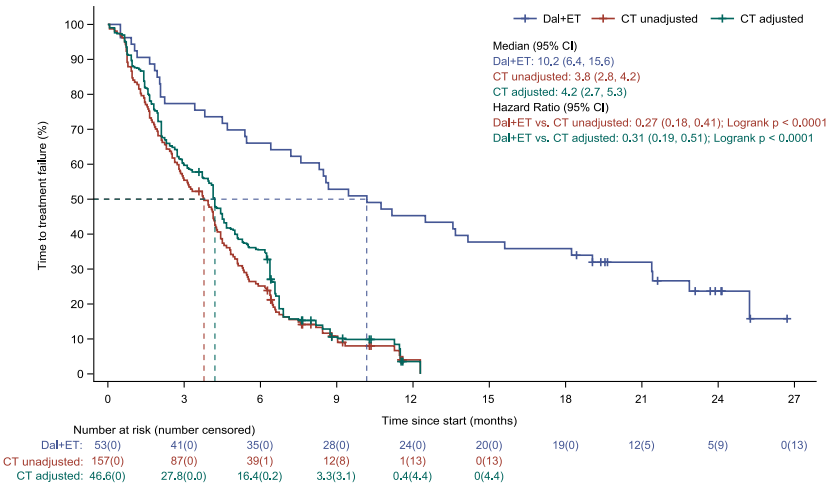

Extended Data Fig. 2 | See next page for caption.

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Extended Data Fig. 2 | Kaplan-Meier survival curves for outcomes between dalpiciclib plus endocrine therapy (Dal+ET) and chemotherapy (CT) groups. (**a**) duration of disease control (DDC), (**b**) progression-free survival (PFS), and (**c**) time to treatment failure (TTF), comparing Dal+ET and CT groups before and after inverse probability of treatment weighting (IPTW) adjustment to estimate the average treatment effect on the treated. IPTW was applied using baseline covariates including age, Eastern Cooperative Oncology Group (ECOG) performance status, disease status (de novo vs. relapse), number of metastatic sites, prior lines of therapy, and type of visceral crisis. Time-to-event outcomes were analyzed using two-sided log-rank tests, and hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated using Cox proportional hazards models. No adjustments were made for multiple comparisons. Dal+ET, dalpiciclib plus endocrine therapy; CT, chemotherapy; CI, confidence interval; HR, hazard ratio; NE, not estimable.

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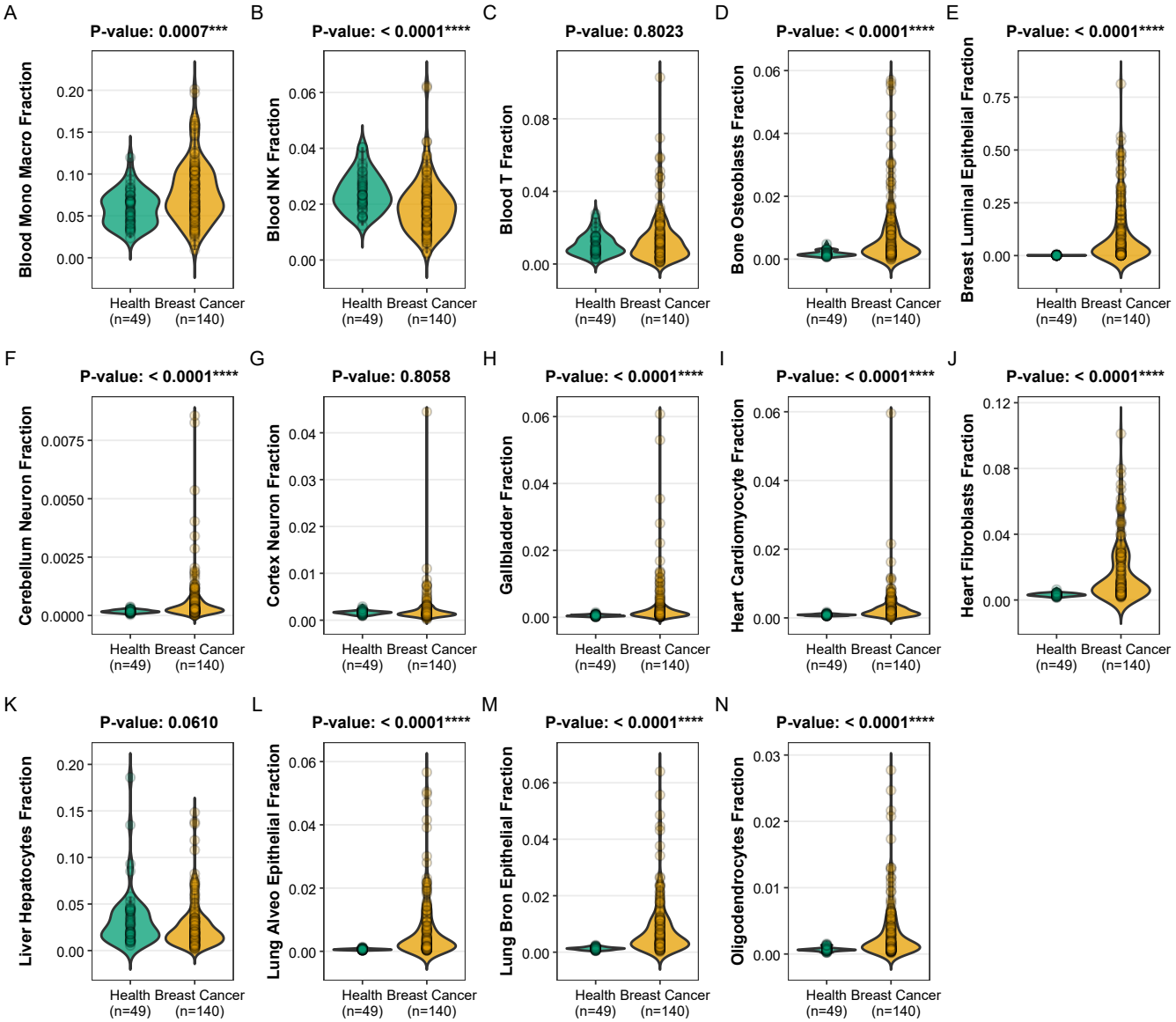

Extended Data Fig. 3 | Distinct cfDNA methylation profiles between breast cancer patients and healthy individuals. Panels **a**-**n** show cfDNA fractions assigned to monocyte/macrophage, NK cell, T cell, bone osteoblast, breast luminal epithelial, cerebellum neuron, cortex neuron, gallbladder, heart cardiomyocyte, heart fibroblast, liver hepatocyte, lung alveolar epithelial, lung bronchial epithelial and oligodendrocyte origins, respectively. n denotes plasma samples from healthy individuals (n = 49) or breast cancer patients (n = 140). Violin plots show the full distribution; each point is one plasma sample. Box plots show the median, 25th and 75th percentiles and whiskers extending to the smallest and largest values within 1.5× the interquartile range. Two-sided Wilcoxon rank-sum tests were used; exact P values were calculated where possible, with normal approximation used when ties precluded exact calculation. Asterisks appended to panel-title P values denote the significance level of the overall comparison in each panel. P values for A-N were P = 0.0007, P < 0.0001, P = 0.8023, P < 0.0001, P < 0.0001, P < 0.0001, P = 0.8058, P < 0.0001, P < 0.0001, P < 0.0001, P = 0.0610, P < 0.0001, P < 0.0001 and P < 0.0001, respectively. **** denotes P < 0.0001.

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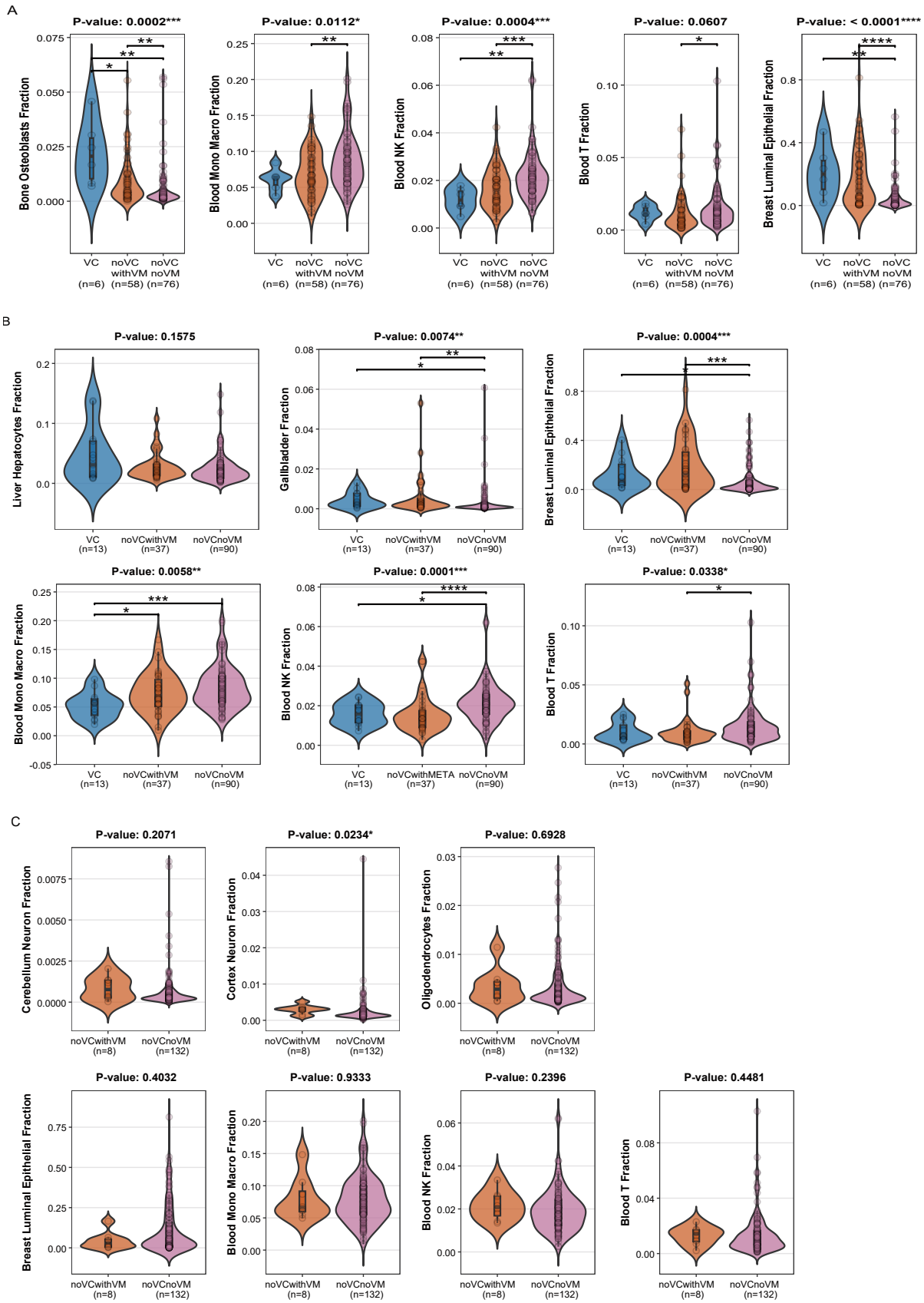

Extended Data Fig. 4 | See next page for caption.

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Extended Data Fig. 4 | CfDNA patterns across bone-related crisis and bone metastasis. (**a**) Bone osteoblast, monocyte/macrophage, NK cell, T cell and breast luminal epithelial cfDNA fractions, respectively, in patients with bone-related crisis (VC, n = 6 plasma samples), bone metastasis without crisis (noVCwithVM, n = 58 plasma samples) or no bone metastasis (noVCnoVM, n = 76 plasma samples). (**b**) Liver hepatocyte, gallbladder, breast luminal epithelial, monocyte/macrophage, NK cell and T cell cfDNA fractions, respectively, in patients with hepatic crisis (VC, n = 13 plasma samples), liver metastasis without crisis (noVCwithVM, n = 37 plasma samples) or no liver metastasis (noVCnoVM, n = 90 plasma samples). (**c**) Cerebellum neuron, cortex neuron, oligodendrocyte, breast luminal epithelial, monocyte/macrophage, NK cell and T cell cfDNA fractions, respectively, in patients with brain metastasis without crisis (noVCwithVM, n = 8 plasma samples) or no brain metastasis (noVCnoVM, n = 132 plasma samples). Violin plots show distributions; each point is one plasma sample. Box plots show the median, 25th and 75th percentiles and whiskers extending to the smallest and largest values within 1.5× the interquartile range. Global three-group comparisons used two-sided Kruskal-Wallis tests. Asterisks appended to panel-title P values denote the significance level of the overall comparison in each panel. Brackets denote exploratory two-sided Wilcoxon rank-sum tests without multiple-comparison adjustment. All reported P values are exact. *, **, *** and **** indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively.

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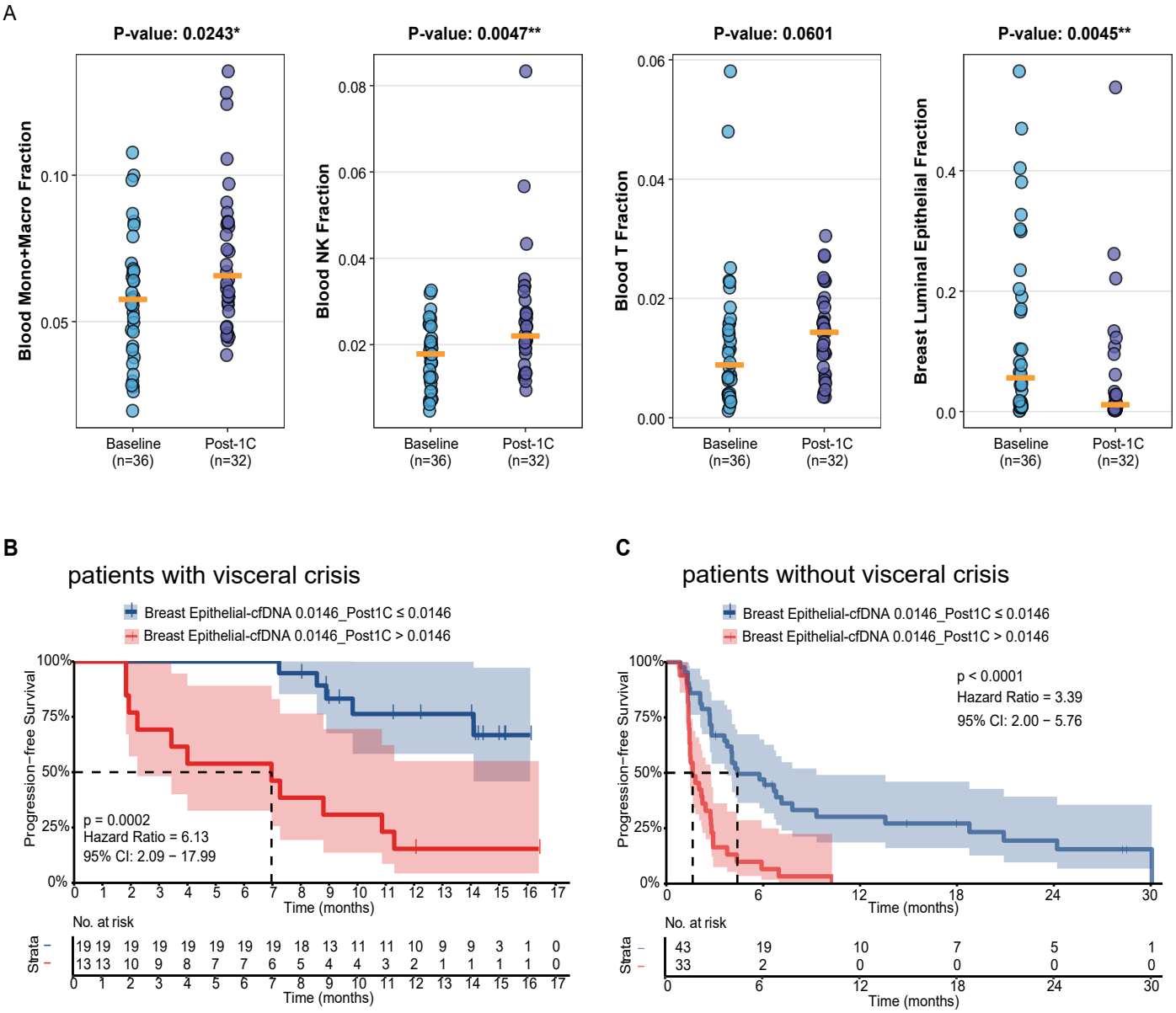

Extended Data Fig. 5 | Dynamic cfDNA changes before treatment and after one cycle of treatment. (**a**) Dynamic cfDNA changes of monocyte/macrophage, NK cell, T cell and breast luminal epithelial cfDNA fractions, respectively, at baseline (n = 36 plasma samples) and after one treatment cycle (Post-1C, n = 32 plasma samples). Points denote individual plasma samples; horizontal crossbars indicate the median. Comparisons were analysed as unpaired two-sided Wilcoxon rank-sum tests without multiple-comparison adjustment. Exact P values were calculated where possible, and the normal approximation was used when exact calculation was precluded by ties. Asterisks appended to panel-title P values denote the significance level of the overall comparison in each panel. * and ** denote P < 0.05 and P < 0.01, respectively. (**b-c**) Post-treatment breast-cell-derived cfDNA stratifies progression-free survival. Kaplan-Meier estimates of progression-free survival are shown for patients with visceral crisis (VC) (B; n = 32 patients) and without VC (C; n = 77 patients) after one cycle of treatment. n denotes patients with post-cycle-1 plasma cfDNA and progression-free survival data. Shaded bands show 95% confidence intervals around Kaplan-Meier estimates, and risk tables show the number of patients at risk. HR was estimated using a Cox proportional hazards model.

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☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data collection was performed using Bioknow EDC.
Data analysis	All statistical analyses were performed using SAS software, version 9.4 or later.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The raw sequence data of cfDNA methylation reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA011705) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human. De-identified individual participant data that underlie the results reported in this article (including baseline characteristics, efficacy outcomes, and safety data), along with aggregated cfDNA methylation profiles, will be made available to qualified researchers upon reasonable request.

Requests for access should be directed to the corresponding author, Dr. Fei Ma (email: drmafei@126.com). Data will be shared for non-commercial academic research purposes only and will require a data sharing agreement with the sponsoring institution. The study protocol and statistical analysis plan are provided alongside this publication. Source data have been provided as Source Data files.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Our study exclusively involves female patients due to its focus on breast cancer.
Reporting on race, ethnicity, or other socially relevant groupings	All patients were Chinese.
Population characteristics	The mean age of patients was 55.8±9.9 years, with the majority (88.7%) having an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Of the cohort, 22.6% had de novo ABC, while 77.4% had relapsed disease. Additionally, 64.2% of patients had not received prior CT for advanced disease. Visceral crisis presentations included effusion in 50.9% of patients, symptomatic crisis in 17.0%, laboratory abnormalities in 22.6%, and 9.4% categorized under other types.
Recruitment	This multicenter, phase II trial was conducted across seven centers in China. Eligible participants were postmenopausal and pre-/perimenopausal women aged ≥18 years, with histologically or cytologically confirmed progesterone or estrogen receptor-positive (ER+ or PR+) and HER2-negative ABC. Patients with measurable disease, as defined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, were included. Candidates for curative surgical resection or radiotherapy were excluded. Participants had to be deemed unsuitable for CT, either due to unwillingness, inability to tolerate CT, or investigator assessment of contraindications. Visceral crisis was defined as the presence of symptoms attributable to symptomatic brain or visceral metastases, with at least one of the following criteria: carcinomatous meningitis or leptomeningeal metastasis; pleural effusion; ascites; abdominal pain due to liver or peritoneal metastasis; dyspnea resulting from pleural effusion; liver enzyme levels >2× the upper limit of normal (ULN); bilirubin levels >1.5× ULN (excluding Gilbert's syndrome or biliary obstruction); pathologically confirmed bone marrow metastasis; or hemoglobin levels <100 g/L. Exclusion criteria included primary resistance to ET (defined as disease recurrence within two years of adjuvant ET or progression within six months of first-line ET in advanced disease), receipt of more than three prior lines of ET, prior treatment with any CDK4/6 inhibitor, and bone-only metastasis.
Ethics oversight	The study protocol was approved by the Ethics Committee of the Cancer Hospital, Chinese Academy of Medical Sciences, and all participating centers. The written informed consent was obtained from all participants.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	This study employed a Simon two-stage design with a one-sided α of 0.1, targeting a statistical power of at least 80%. The null hypothesis posited a 6-month survival rate of 44%, while the alternative hypothesis anticipated a 6-month survival rate of 60% in the Dal+ET group. In the first stage, an initial cohort of 18 evaluable patients was enrolled. If 9 or more patients were alive at 6 months, the study would proceed to the second stage, enrolling an additional 35 patients, for a total of 53 evaluable participants. If more than 27 patients survived at 6 months in the full cohort, the treatment regimen would be considered promising and suitable for further investigation.
Data exclusions	No data were excluded for the analysis.
Replication	Due to limited sample of tumor biopsy, we only performed biomarker analysis once.
Randomization	This is a single-arm study, so randomization was not performed.
Blinding	This is a single-arm study, so blinding is not application.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	The study was registered prior to patient enrollment (NCT05431504).
Study protocol	The trial protocol is available in the Supplementary file.
Data collection	Between February 2023 and February 2024, 53 patients with visceral crisis were enrolled in the Dal+ET group. The data were collected at all participating centers.
Outcomes	The primary endpoint was the 6-month survival rate, defined as the proportion of patients alive at 6 months following treatment initiation. Secondary endpoints included overall survival (OS), PFS, time to treatment failure (TTF), the 3-month treatment failure rate (TFR), duration of disease control (DDC), objective response rate (ORR), disease control rate (DCR), and safety. Detailed definitions of these endpoints are provided in the study protocol. Biomarker analysis was performed to assess the molecular characteristics of plasma cfDNA in patients with or without visceral crisis.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*