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ORIGINAL ARTICLE

An open-label, phase IB/II study of abemaciclib with paclitaxel for tumors with *CDK4/6* pathway genomic alterations

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Available online xxx

Background: Disruption of cyclin D-dependent kinases (CDKs), particularly *CDK4/6*, drives cancer cell proliferation via abnormal protein phosphorylation. This open-label, single-arm, phase Ib/II trial evaluated the efficacy of the *CDK4/6* inhibitor, abemaciclib, combined with paclitaxel against *CDK4/6*-activated tumors.

Patients and methods: Patients with locally advanced or metastatic solid tumors with *CDK4/6* pathway aberrations were included. Based on phase Ib, the recommended phase II doses were determined as abemaciclib 100 mg twice daily and paclitaxel 70 mg/m^2 on days 1, 8, and 15, over 4-week-long cycles. The primary endpoint for phase II was the overall response rate (ORR). The secondary endpoints included the clinical benefit rate (CBR), progression-free survival (PFS), overall survival (OS), and safety. Tissue-based next-generation sequencing and exploratory circulating tumor DNA analyses were carried out.

Results: Between February 2021 and April 2022, 30 patients received abemaciclib/paclitaxel (median follow-up: 15.7 months), and 27 were included in the efficacy analysis. *CDK4/6* amplification (50%) and *CCND1/3* amplification (20%) were common activating mutations. The ORR was 7.4%, with two partial responses, and the CBR was 66.7% (18/27 patients). The median OS and PFS were 9.9 months [95% confidence interval (CI) 5.7-14.0 months] and 3.5 months (95% CI 2.6-4.3 months), respectively. Grade 3 adverse events (50%, 21 events) were mainly hematologic. Genetic analysis revealed a 'poor genetic status' subgroup characterized by mutations in key signaling pathways (RAS, Wnt, PI3K, and NOTCH) and/or *CCNE* amplification, correlating with poorer PFS.

Conclusion: Abemaciclib and paclitaxel showed moderate clinical benefits for *CDK4/6*-activated tumors. We identified a poor genetic group characterized by bypass signaling pathway activation and/or *CCNE* amplification, which negatively affected treatment response and survival. Future studies with homogeneous patient groups are required to validate these findings.

Key words: *CDK4/6*, abemaciclib, paclitaxel

INTRODUCTION

Cyclin D-dependent kinases (CDKs) and cyclins, including cyclin D family CCND1, CCND2, and CCND3, are often activated in human cancers, affecting the cell cycle progression by driving the G1-to-S phase transition.[1] These proteins form complexes with *CDK4/6*, leading to phosphorylation of retinoblastoma (Rb) proteins and activation of E2F transcription factors, which promote DNA replication.[2] In many cancers, amplification or overexpression of *CCND1/2/3* or *CDK4/6* accelerates this process, resulting in excessive Rb phosphorylation, loss of checkpoint integrity, and uncontrolled cell proliferation.[3] Data from The Cancer Genome Atlas indicate that such alterations, along with changes in other cell-cycle-related genes like *CCNE1, CDKN2A, CDKN2B*, or *Rb*, are observed in ∼15%-30% of solid cancers, including sarcoma, glioblastoma, melanoma, germ cell tumors, and certain gynecological cancers.[4-6]

The dysregulation of the cyclin D-*CDK4/6* pathway is associated with poor prognosis and treatment resistance in various cancers.[7] However, tumors with these alterations may also exhibit increased sensitivity to *CDK4/6* inhibitors, presenting a promising strategy for targeted therapies.[8] As cyclin D and CDKs are intricately regulated within the cell cycle machinery, targeting these cell cycle proteins has emerged as a compelling approach to impede tumor growth.[9]

To implement this strategy, *CDK4/6* inhibitors, namely abemaciclib, ribociclib, and palbociclib, have been among

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the most heavily investigated and successful drug groups.[10,11] Abemaciclib, an oral, selective, ATP-competitive *CDK4/6* inhibitor, has shown notable success in clinical trials. When administered continuously at a dose of 150 mg twice daily in combination with endocrine therapy for hormone receptor-positive, human epidermal growth factor receptor 2-negative (HR+/HER2−) breast cancer, abemaciclib improved overall survival (OS) and progression-free survival (PFS), while showing an acceptable safety profile in two phase III trials.[12,13] Therefore, the US Food and Drug Administration approved abemaciclib for use in adjuvant and palliative settings. Furthermore, an ongoing phase II basket trial is currently evaluating the efficacy of abemaciclib monotherapy in patients with genomic alterations in *CCND1/2/3* or *CDK4/6* (NCT03310879).

Although *CDK4/6* inhibitor monotherapy has shown antitumor activity, the response rate remains modest (<5%).[14,15] However, combinations of *CDK4/6* inhibitors with targeted therapies or cytotoxic chemotherapeutic agents hold promise for exerting a synergistic effect.[16,17] A preclinical study using lung adenocarcinoma cells showed a synergistic effect of a *CDK4/6* inhibitor and paclitaxel combination, regardless of KRAS mutations, suggesting this combination as a potential treatment option.[18] The use of paclitaxel has been explored in various solid cancers, including sarcoma and breast, gastric, and prostate cancers, showing its potential as a combinatorial agent.[19] A recent phase I study evaluated the feasibility and safety of combining the *CDK4/6* inhibitor palbociclib with paclitaxel in breast cancer patients.[20] However, no studies have explored the efficacy and safety of *CDK4/6* inhibitor and chemotherapy combination against a specific biomarker-driven cohort.

Therefore, based on the potential synergistic effects of abemaciclib and paclitaxel, which cause G1 arrest and M-phase derangement of the cell cycle, respectively,[18] we conducted an open-label phase Ib/II trial to evaluate the efficacy of abemaciclib combined with paclitaxel in *CDK4/6* pathway-activated tumors. Additionally, we carried out next-generation sequencing (NGS) using pretreatment tissue biopsy and circulating tumor DNA (ctDNA) to correlate clinical outcomes with molecular and genomic biomarkers to identify patients most likely to benefit from this combination.

PATIENTS AND METHODS

Patient selection

Eligible patients had histologically or cytologically confirmed locally advanced or metastatic solid tumors with *CDK4/6* pathway aberrations, including *CCND1/2/3* or *CDK4/6* amplification, *CCND1* mutation, or *CCND1* splice mutation detected by the NGS or fluorescence *in situ* hybridization analyses. Other eligibility criteria included age ≥19 years and having an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; a measurable or evaluable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1[20,21]; and adequate bone marrow, renal, and hepatic functions. Major exclusion criteria included uncontrolled brain and central nervous system metastases, uncontrolled major cardiovascular disease, and prior exposure to abemaciclib.

Trial design and study procedure

Phase Ib of the study (ClinicalTrials.gov: NCT04594005) aimed to assess the safety and tolerability of the abemaciclib and paclitaxel combination, and the standard 3 + 3 design with a starting dose (dose level 1) and dose levels −1 and −2 was adopted. Dose level 1 was as follows: abemaciclib 100 mg twice daily and paclitaxel 80 mg/m^2 on days 1, 8, and 15 of each 4-week cycle. For dose level −1, the paclitaxel dose was reduced to 70 mg/m^2, and for dose level −2, the abemaciclib dose was lowered to 50 mg twice daily with a paclitaxel dose of 70 mg/m^2. Dose-limiting toxicities (DLTs) were assessed during the first 4 weeks (the 28 days after day 1 dose), and the recommended phase II dose (RP2D) was determined (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2024.104106).

Then, the study progressed to phase II with a total of 30 patients, including those who received doses equal to or higher than the final RP2D in phase Ib. Tumor assessments were carried out following RECIST version 1.1 at 8-week intervals during the 16 weeks after the first dose, followed by assessments every 12 weeks thereafter until radiographic documentation of progressive disease. Treatment was continued until disease progression, intolerable toxicity, or withdrawal of informed consent, whichever occurred first.

Study design and statistical analysis

The primary endpoint of phase II was the overall response rate (ORR), which included the rates of complete response (CR) and partial response (PR) (determined according to RECIST version 1.1) as the best response. The secondary endpoints included the clinical benefit rate (CBR), defined as the percentage of patients achieving CR, PR, or stable disease (SD) as the best response; duration of response; PFS; OS; and safety profiles.

The sample size was determined using the minimax two-stage design to evaluate a null hypothesis of ORR <5% versus an alternative hypothesis of ORR ≥20%, with $\alpha = 0.05$ and $\beta = 0.2$. If one or more successes were observed in the initial 13 patients, recruitment continued to a total of 27 patients. If four or more responses were observed, additional investigation was warranted. Accounting for a 10% dropout rate, the total sample size, including phase Ib patients who received the RP2D, was calculated as 30 patients.

The efficacy set was defined as patients who had received a dose equal to or higher than the final RP2D and had undergone at least one response evaluation besides the baseline. Safety was assessed in patients who received at least one dose of the study drug. Adverse events (AEs) were graded and recorded per the National Cancer Institute Common Terminology Criteria for Adverse Events

(NCI-CTCAE), version 4.3. All tests were two-sided, with a significance level of $P < 0.05$.

Correlative science

For the tissue-based tumor NGS analysis, genomic DNA was isolated from formalin-fixed paraffin-embedded samples. Targeted DNA sequencing was carried out using the high-throughput TruSight Oncology 500 platform (Illumina, San Diego, CA). In cases where patients had previously received tumor NGS reports from other platforms, the findings from these reports were utilized.

For the ctDNA analyses, blood samples were collected at baseline, at week 8, and within 28 days of disease progression. For targeted panel sequencing, a DNA NGS library was constructed, and solution-based target enrichment was carried out using the AlphaLiquid® 100 target capture panel (IMBdx, Inc., Seoul, South Korea), including 118 cancer-related genes and covering the entire gene exons. The captured DNA libraries were sequenced using the NextSeq 550Dx platform (Illumina). In both analyses, clinically significant variants identified in the NGS reports were used.

Ethics approval and consent to participate

This study was conducted according to the ethical principles for medical research involving human subjects stated in the Declaration of Helsinki and the ICH Good Clinical Practice guidelines. The study protocol was reviewed and approved by the institutional review board of the participating center. The study, timelines, and outcome measures were explained to all eligible participants. Participants were informed that they were free to discontinue their participation at any time without any consequences. All the participants provided written informed consent.

RESULTS

Patients

Between February 2021 and April 2022, 30 patients were enrolled in phases Ib ($n = 6$) or II ($n = 24$), of whom 27 underwent radiographic imaging and were consequently included in the efficacy analysis (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2024.104106). Table 1 shows the patients' baseline clinical characteristics and demographics. The most common *CDK4/6* pathway-activating mutations were *CDK4/6* amplification (15 patients, 50%) and *CCND1/3* amplification (6, 20%). Most patients (17 patients, 56.7%) had received two or more prior lines of systemic therapy.

Treatment efficacy

In phase Ib, two of three patients experienced grade 3 and 4 neutropenia as DLTs at dose level 1, and three additional patients were enrolled at dose level −1. No DLTs or unexpected toxicities occurred at this level, and the RP2D was determined as paclitaxel 70 mg/m^2 with abemaciclib 100 mg twice daily, as shown in Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2024.104106.

Table 1. Baseline characteristics

Total ($N = 30$)	No. of patients	(%)
Median age (range), years	60 (20-72)	
Gender		
Male	15	50.0
Female	15	50.0
Tumor type		
Soft tissue sarcoma	13	43.3
Stomach[a]	5	16.7
Melanoma	3	10.0
Head and neck[b]	3	10.0
Thyroid	2	6.7
Lung	1	3.3
Breast	1	3.3
Duodenal	1	3.3
Cervix	1	3.3
Renal pelvis	1	3.3
Mutation subgroup		
CDK4 amplification	13	43.3
CDK6 amplification	2	6.7
CCND1 amplification	4	13.3
CCND3 amplification	2	6.7
CCND3 mutation	1	3.3
CDKN2A amplification	2	6.7
CDKN2A/CDKN2B loss	1	3.3
CDKN2C mutation	1	3.3
MDM2 amplification	3	10.0
MEN1, *TSC2* mutation	1	3.3
Stage at diagnosis		
I	4	13.3
II	5	16.7
III	11	36.7
IV	10	33.3
ECOG performance status		
0	18	60.0
1	12	40.0
Prior radiotherapy		
Yes	15	50.0
No	15	50.0
Prior surgery		
Yes	25	83.3
No	5	16.7
No. of prior chemotherapy		
0	2	6.7
1	11	36.7
2	7	23.3
3	3	10.0
≥4	7	23.3

ECOG, Eastern Cooperative Oncology Group.
[a]Stomach including adenocarcinoma and neuroendocrine carcinoma.
[b]Head and neck cancers: salivary gland cancer and tongue cancer.

At the data cut-off date (31 March 2023), one patient continued treatment with a median follow-up duration of 15.7 months [95% confidence interval (CI) 11.1-25.4 months]. Of the 27 assessable patients in the efficacy set, 2 patients (7.4%) achieved PR, and 16 (59.3%) had SD, yielding an ORR of 7.4% and a CBR of 66.7% (Table 2). The median OS and PFS were 9.9 months (95% CI 5.7-14.0 months) and 3.5 months (95% CI 2.6-4.3 months), respectively (Figure 1A and B).

Tumor shrinkage was observed in 33.3% (8/24) of patients with measurable target lesions, with one breast cancer patient and one gastric cancer patient achieving target lesion shrinkage of −41.6% and −30.8%, respectively. Figure 1C shows a waterfall plot illustrating the best tumor shrinkage from baseline. The effects of the

Table 2. Efficacy results

Response evaluation	Patients (N = 27) No.	%
Overall response rate[a]	2	7.4
Complete response	0	0.0
Partial response	2	7.4
Stable disease	16	59.3
Progressive disease	9	33.3
Not available	3	
Clinical benefit rate[b]	18	66.7

[a]Overall response rate includes complete response and partial response.
[b]Clinical benefit rate, or disease control rate, includes complete response, partial response, and stable disease.

abemaciclib and paclitaxel combination grouped according to tumor subtype are shown in a swimmer plot (Figure 1D). Prolonged PFS (>6 months) was observed in nine patients (33.3%) with soft tissue sarcoma (STS; five SD), gastric cancer (one PR, one SD), melanoma (one SD), and breast cancer (one PR).

AEs and tolerability

Among the 30 patients, AEs of any cause led to dose reduction of abemaciclib to 50 mg in 9 patients (30.0%) and discontinuation in 1 (3.3%). Paclitaxel discontinuation or dose reduction to 60 mg or 50 mg occurred in 2 (6.7%), 10 (33.3%), and 3 (10.0%) patients, respectively. The mean (±standard deviation) dose intensities were 79.2% (±37.2%) for abemaciclib and 76.1% (±22.5%) for paclitaxel.

Hematologic toxicities were the most common AEs, with anemia in 19 (63.3%) patients, followed by neutropenia, leukopenia, and thrombocytopenia in 18 (60.0%), 9 (30.0%), and 3 (10.0%) patients, respectively. Other non-hematologic AEs included diarrhea (36.7%), increased creatinine concentration (20%), and neuropathy (6%). Grade 3 or higher treatment-related AEs occurred in 15 patients (50%, total: 21 events), which were mainly hematologic (19 events). No treatment-related deaths occurred during the study (Table 3).

Genomic landscape and association with clinical efficacy

All patients included in the efficacy analysis had available targeted tumor NGS data (Figure 2A). The most common mutated gene was *TP53* in nine patients (Supplementary Appendix 1, available at https://doi.org/10.1016/j.esmoop.2024.104106), although *TP53* mutation status was not

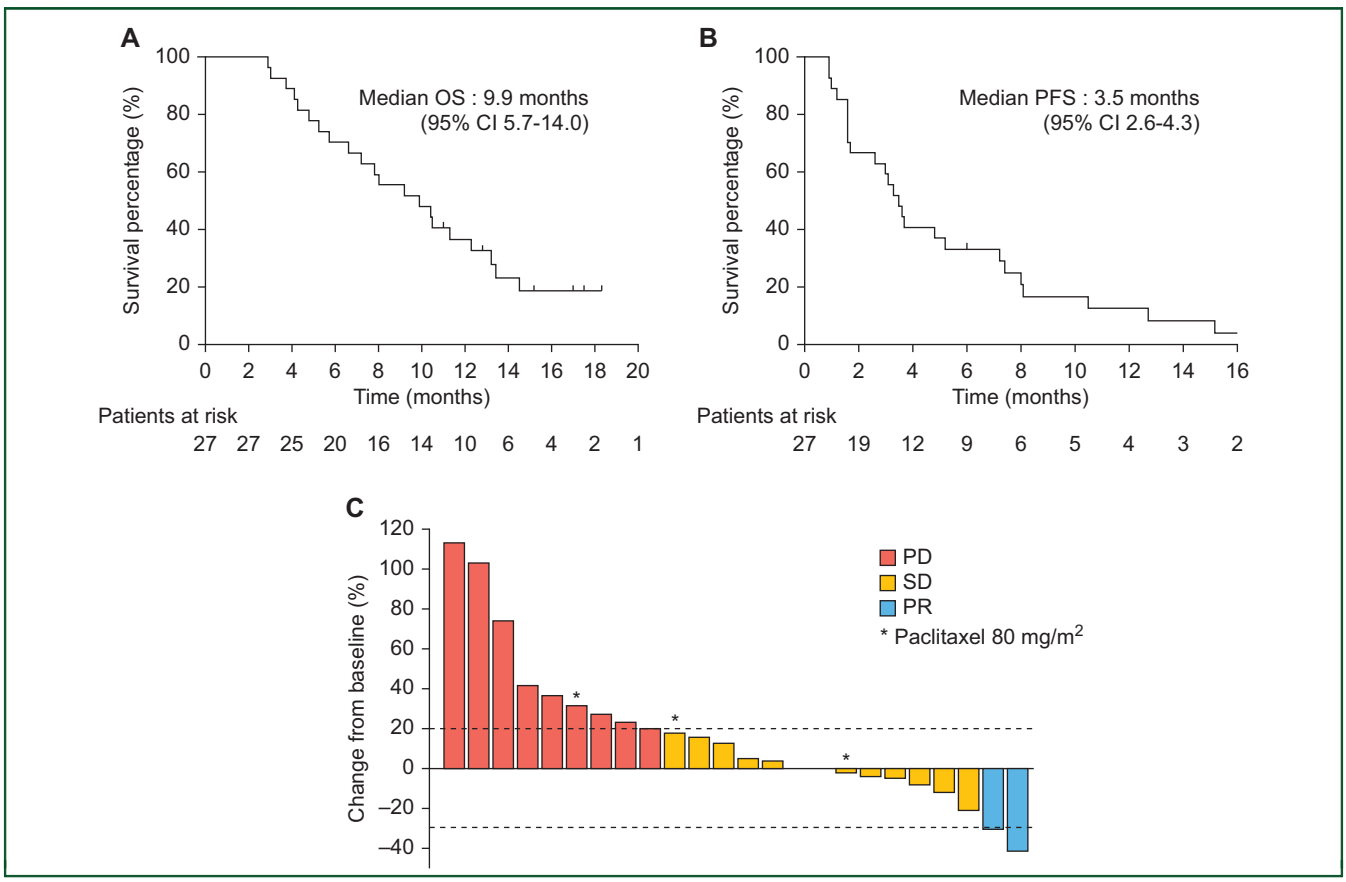

Figure 1. Survival outcome and efficacy of abemaciclib + paclitaxel. (A) Overall survival. (B) Progression-free survival. (C) Waterfall plot showing best tumor response from baseline; three patients without measurable target lesions at baseline are not represented in the graph. Patients who received 80 mg/m^2 of paclitaxel in phase Ib are marked with an asterisk (*). (D) Swimmer plot according to cancer type. Each bar represents one patient, and the bar length represents the progression-free period. Boxes in the left column show clinical characteristics and key mutations in the *CDK4/6* pathway, as well as whether the patient features a poor genetic status, defined as either *CCNE* amplifications and/or mutations in the key signaling pathways.
CDK, cyclin D-dependent kinase; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

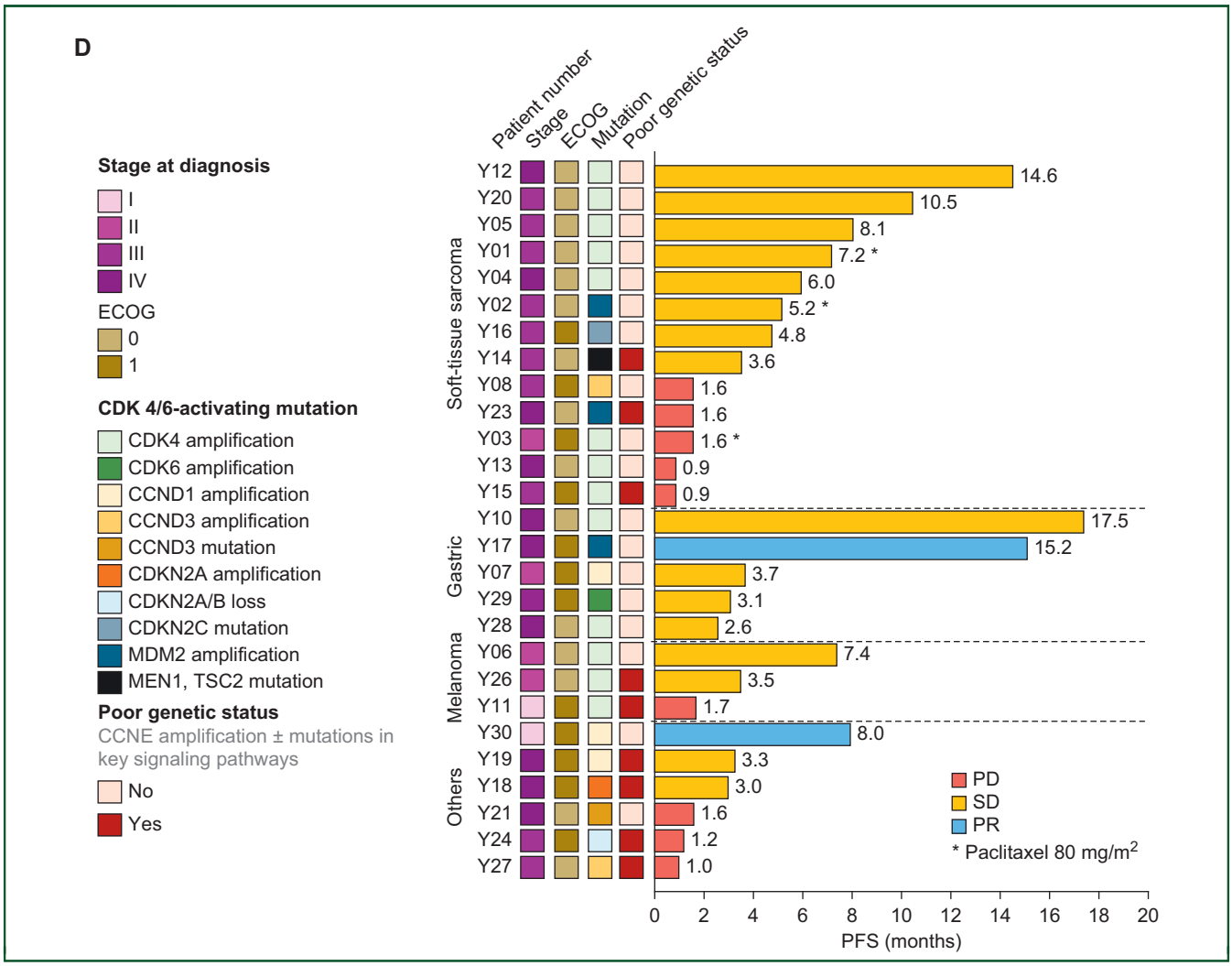

Figure 1. Continued.

associated with PFS [median PFS 3.3 months (95% CI 3.0 months-NA) for patients with mutations versus 3.5 months (95% CI 1.6-10.5 months) for wild-type patients; $P = 0.80$].

Regarding mutations related to key signaling pathways (Figure 2A), we considered several genes as belonging to certain signaling pathways: *NF1*, *HRAS*, *RASA1*, and *MED12* to the RAS signaling pathway ($n = 5$); *MEN1* and *FAT1* to the Wnt signaling pathway ($n = 2$); *TSC2*, *PIK3R1*, *STK11,* and *PIK3CA* to the PI3K signaling pathway ($n = 4$); and *NOTCH4* to the NOTCH signaling pathway ($n = 1$). Mutations within each signaling pathway tended to correlate with poor PFS but without statistical significance (Supplementary Figure S3A, available at https://doi.org/10.1016/j.esmoop.2024.104106). However, patients harboring mutations in any of these signaling pathways ($n = 8$) showed significantly worse PFS [median PFS 2.4 months (95% CI 1.6 months-NA) for patients with mutations versus 5.2 months (95% CI 3.1-10.5 months) for those without ($n = 19$); $P = 0.007$; Figure 2B].

For copy number alterations, *CCNE* amplification was a significant key determinant of PFS [median PFS 0.9 months (95% CI 0.9 months-NA) for *CCNE*-amplified patients ($n = 2$) versus 3.6 months (95% CI 3.0-8.0 months) for those without *CCNE* amplification; $P < 0.001$; Figure 2C]. However, none of the *CDK4/MDM2*, *CDK6*, *CCND1*, or *CCND3* amplifications demonstrated statistical significance (Supplementary Figure S3B, available at https://doi.org/10.1016/j.esmoop.2024.104106).

Given these findings, we defined poor genetic status as harboring a mutation in bypass signaling pathways and/or *CCNE* amplification. Accordingly, patients were divided into poor and favorable groups. Compared with the favorable genetic status group ($n = 18$), the poor genetic status group ($n = 9$) showed significantly worse PFS [median PFS 1.7 months (95% CI 1.2 months-NA) versus 6.2 months (95% CI 3.1 months-NA) for the favorable group; $P = 0.001$; Figure 2D]. This association was consistently significant in the multivariate Cox proportional hazard model analysis after adjusting for tumor type (Figure 2E). In our study, patients with STS showed minimal activation of these bypass signaling pathways and/or *CCNE* amplification relative to other tumor types, and while survival differences across tumor subgroups were not statistically significant, STS patients exhibited a trend toward longer survival

Table 3. Treatment-related adverse events

	All grades		Grade 3	
	No.	%	No.	%
Hematologic				
Anemia	19	63.3	1	3.3
Neutropenia	18	60.0	10	33.3
Leukopenia	9	30.0	6	20.0
Thrombocytopenia	3	10.0	2	6.7
Non-hematologic				
Diarrhea	11	36.7	0	0.0
Creatinine elevation	6	20.0	0	0.0
Neuropathy	6	20.0	0	0.0
Rash	5	16.7	0	0.0
General weakness	5	16.7	0	0.0
Nausea	4	13.3	0	0.0
Edema	4	13.3	0	0.0
Anorexia	4	13.3	0	0.0
Abdominal pain	3	10.0	0	0.0
Dyspnea	2	6.7	0	0.0
Infection	2	6.7	0	0.0
ALP elevation	2	6.7	0	0.0
Cough	2	6.7	0	0.0
Constipation	2	6.7	0	0.0
AST elevation	2	6.7	1	3.3
Pneumonitis	2	6.7	0	0.0
Vomiting	2	6.7	0	0.0
Dyspepsia	2	6.7	0	0.0
Heartburn	2	6.7	0	0.0
Urine discoloration	2	6.7	0	0.0
Tumor necrosis	1	3.3	1	3.3
Pruritus	1	3.3	0	0.0
ALT elevation	1	3.3	0	0.0
Back pain	1	3.3	0	0.0
Hypertension	1	3.3	0	0.0
Mucositis/stomatitis	1	3.3	0	0.0
Asthenia	1	3.3	0	0.0
Paronychia	1	3.3	0	0.0
Fever	1	3.3	0	0.0
Pneumothorax	1	3.3	0	0.0
Hemorrhoid	1	3.3	0	0.0

ALP, alkaline phosphatase.

(Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.104106).

Exploratory ctDNA analysis

Baseline and follow-up ctDNA results were available for 20 patients in the efficacy set (Figure 3A and Supplementary Appendix 2, available at https://doi.org/10.1016/j.esmoop.2024.104106). Interestingly, three patients acquired mutations related to bypass signaling pathways that were not present initially: *GNAQ* R183Q (patient Y13), *TSC2* R611W (patient Y14), and *PIK3CA* H1047R (patient Y18), all of which were detected upon disease progression (Figure 3B-D). Notably, the patient with *GNAQ* R183Q (Y13 patient) at the end of the trial did not exhibit poor genetic status at baseline evaluation.

DISCUSSION

In this phase Ib/II trial, the abemaciclib and paclitaxel combination showed a clinical benefit of 66.7% in *CDK4/6* pathway-activated solid tumors. To our knowledge, this is the first study to combine a *CDK4/6* inhibitor with a chemotherapeutic agent in patients with tumors harboring genetic alterations in the targeted pathway. We also evaluated the relationship between clinical outcomes and the *CDK4/6* inhibitor combination through integrative molecular analysis.

Recent advancements in genomic profiling have prompted investigations into personalized medical strategies based on molecular characteristics. Following evidence of the benefits of *CDK4/6* inhibitors in hormone-responsive breast cancer and dedifferentiated liposarcoma (DDLPS),[22-24] *CDK4/6* inhibitors have recently been evaluated as monotherapy in solid tumors harboring cell cycle-gene aberrations. In the Lung Master Protocol (Lung-MAP) sub-study of squamous cell lung cancer with cell cycle-gene alterations, palbociclib monotherapy yielded an ORR of 6% and a disease control rate of 44%.[25] In another phase II tissue/site-agnostic study in the Signature Program involving tumors with *CDK4/6* pathway alterations, ribociclib monotherapy yielded an ORR of 2.9% and a PFS of 1.8 months.[15]

Despite recent target-enriched treatment approaches, drug activity remains moderately effective. Therefore, based on the synergistic effects of both drugs (abemaciclib causing G1 arrest and paclitaxel affecting M-phase derangement) and preclinical findings,[18] this trial was designed to evaluate the efficacy, safety, and biomarkers associated with this combination. Although cross-study comparisons are suboptimal, the ORR (7.4%) and PFS (3.5 months) observed in our study were favorable compared with those from the Signature Program (ORR 2.9%; PFS 1.8 months).[15] Additionally, considering that most of our study participants were heavily pretreated (56.6% had received two or more lines of prior chemotherapy), our study has yielded comparable clinical benefits lasting 16 weeks or longer in 11 patients (40.7%).

In our study, treatment-related toxicities were generally manageable with dose modifications and appropriate supportive care. We used low-dose weekly paclitaxel to improve patient prognosis and safety, as shown in several randomized trials.[26] Therefore, the rates of grade 3-4 hematologic toxicities, including neutropenia (33.3%), thrombocytopenia (6.7%), and anemia (3.3%), in our study were comparable to or even lower than those reported for palbociclib as monotherapy (50%, 30%, and 17%, respectively) or combined with letrozole (54%, 2%, and 6% in the PALOMA trial).[14,27] Furthermore, compared with phase I trials combining abemaciclib with cytotoxic agents, our study showed markedly lower rates of grade 3-4 hematologic toxicities than those with pemetrexed (neutropenia, 65%; anemia, 26%; thrombocytopenia, 17%) or gemcitabine (neutropenia, 54%; thrombocytopenia, 46%; anemia, 42%). Regarding abemaciclib dose, the mean steady-state area under the concentration-time curve from 0 to 24h ($AUC_{0\text{-}24,ss}$) and the steady-state maximum concentration of the drug in plasma ($C_{max,ss}$) showed consistent levels of more than ~2000 ng*h/ml and ~200 ng/ml, respectively, at dosages of 100 mg, 150 mg, and 200 mg twice daily.[24] In a recent phase I trial of abemaciclib combined with irinotecan and temozolomide, reasonable and stable drug

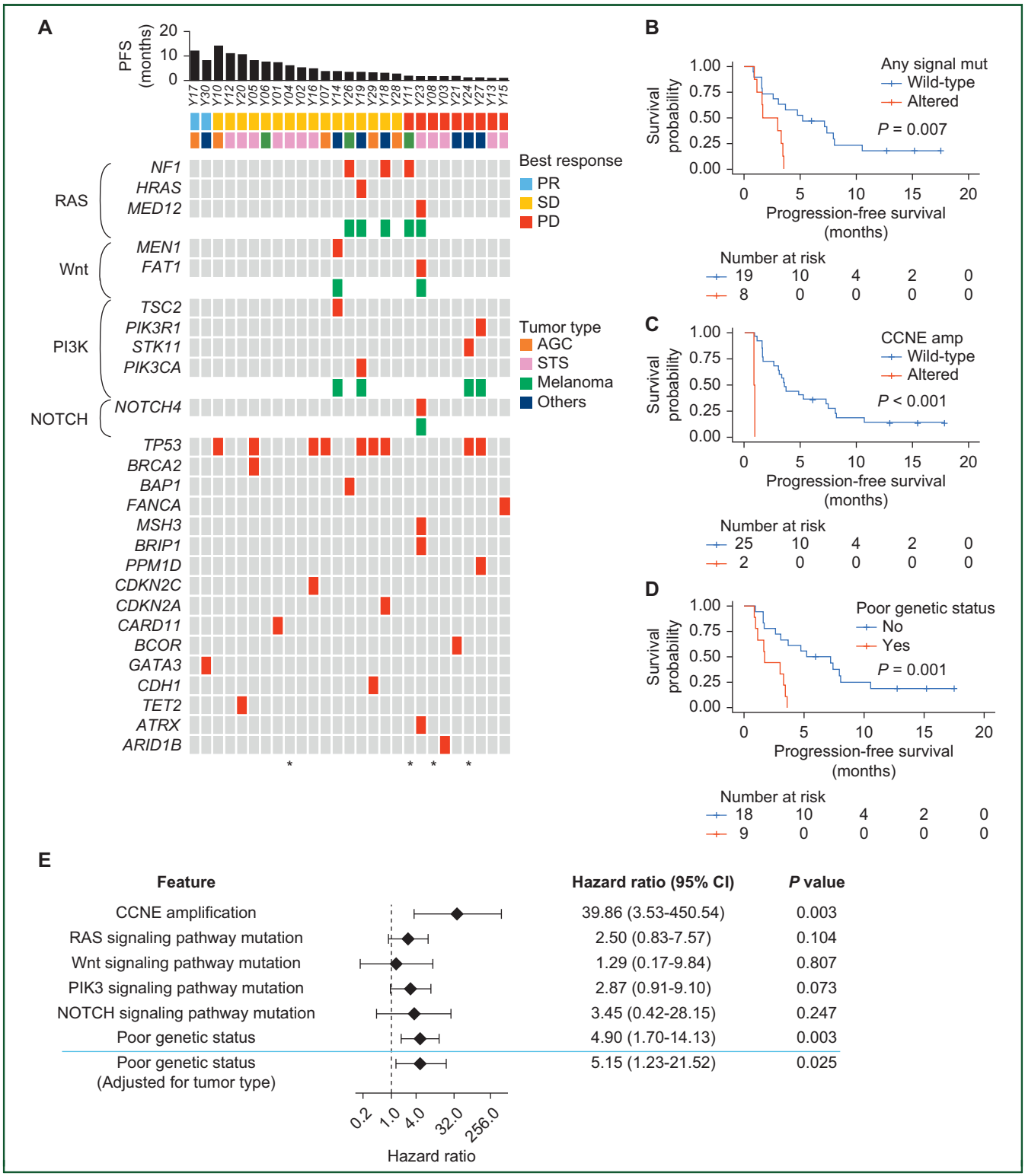

Figure 2. Tumor NGS profiles associated with efficacy. (A) Tumor NGS mutational landscape. The PFS of each patient is shown in the upper bar graph. Each column in the plot represents each patient, and the corresponding best response and tumor type are shown. Each row represents each gene, and the related signaling pathways are marked on the left side of the plot. Asterisks at the bottom represent patients with NGS results from platforms other than TruSight Oncology 500. (B) PFS according to signaling mutation status. (C) PFS according to *CCNE* amplification status. (D) PFS according to poor genetic status. (E) Forest plot showing the results of Cox proportional hazard model analysis evaluating PFS according to genetic features compared with wild-type tumors.
AGC, advanced gastric cancer; CI, confidence interval; NA, not available; NGS, next-generation sequencing; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; STS, soft tissue sarcoma.

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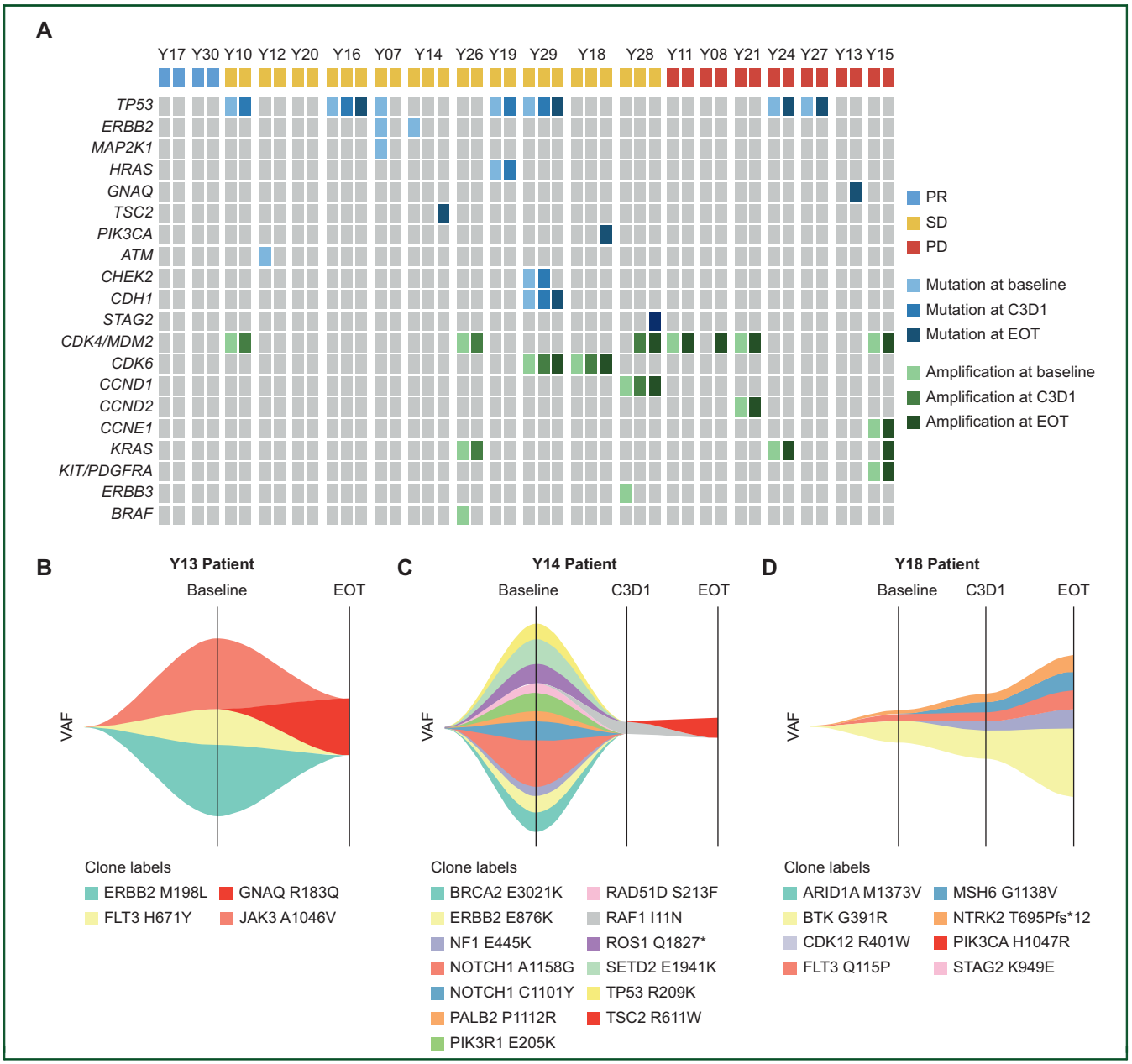

Figure 3. Circulating tumor DNA profiles associated with efficacy. (A) Landscape of serial follow-up of circulating tumor DNA (ctDNA) results. Each section of columns represents each patient. The first column in each section represents the baseline result, and the subsequent column represents the ctDNA result at C3D1 or EOT. Each row represents each gene. (B) Serial follow-up results of variant allele frequency detected in the ctDNA of patient Y13, (C) patient Y14, and (D) patient Y18. Potential bypass-resistant mutations are colored in red.
C3D1, cycle 3 day 1; CB, clinical benefit; EOT, end of treatment; PD, progressive disease; PR, partial response; SD, stable disease; VAF, variant allele frequency.

concentrations (average ∼150 ng/ml) were achieved with 55 mg/m^2 abemaciclib.[28]

Given the aforementioned evidence, this phase Ib study, despite not including a pharmacokinetic analysis, provides crucial insights for identifying the optimal dose of abemaciclib that can effectively and safely act alongside cytotoxic chemotherapy. Similarly, in another study, a lower palbociclib dose (RP2D: 75 mg), combined with paclitaxel, was identified as an option, as opposed to the typical 125 mg dose used in monotherapy.[20] To improve treatment outcomes with manageable side-effects, we propose considering weekly schedules in future trials.

Despite the promising efficacy, there is limited understanding of the mechanisms underlying treatment response, and biomarkers for identifying patients who may benefit from *CDK4/6* inhibitors are urgently needed. Furthermore, although *CDK4/6* inhibitors are the standard treatment for breast cancer, they are currently administered without marker selection other than hormone receptor positivity.[13,29-32]

Few studies have investigated the prognostic importance of the immune microenvironment and potential markers related to various aspects such as cell cycle regulation, oncogenic signaling, DNA damage response/repair

deficiencies, and microenvironment-related signaling.[32-34] Although translational analyses have been conducted in randomized clinical trials of breast cancer,[35-38] reliable markers for predicting clinical outcomes have not been established.

In our basket trial, by including patients with activated *CDK4/6* signaling, as identified by NGS, we narrowed down the starting point of biomarker evaluation. As cyclin E operates downstream of *CDK4/6*,[39] *CCNE* amplification serves as a resistance mechanism against *CDK4/6* inhibitors.[40] Moreover, mutations in *AKT1* and the *RAS* family of oncogenes that provoke drug resistance *in vitro* have been detected in *CDK4/6* inhibitor-resistant cases.[35] Our study suggests that patients with a poor genetic status (*CCNE* amplification and oncogenic bypass signaling pathways) may not benefit from *CDK4/6* inhibitors and highlights the importance of carrying out a comprehensive baseline genomic feature assessment, which requires reliable tumor NGS profiling and may include ctDNA monitoring, allowing for additional spatial and temporal evaluation of tumor heterogeneity and evolution.[41,42]

Our study has a few limitations. Firstly, given its nature as a basket trial, not all tumors were equally represented, potentially leading to heterogeneity in the study population and introducing potential biases. Secondly, the primary objective of the study was not met. However, there were indications of tumor stabilization, particularly in cases with a favorable genomic status. Given that most *CDK4/6* inhibitors have shown low response rates as monotherapy, tumor stabilization is worth considering as a relevant parameter when evaluating *CDK4/6* inhibitor efficacy.[43] Thirdly, because this study was based on a paclitaxel—*CDK4/6* inhibitor combination, whether the identified biomarkers are applicable to *CDK4/6* monotherapy remains uncertain. Finally, the presence of a mutation does not unequivocally confirm bypass signaling pathway activation within tumors. Nonetheless, our approach remains valid, as it facilitates identifying patients who are likely to respond favorably to *CDK4/6* inhibitors, which requires confirmation in future trials.

Conclusion

Although the primary endpoint of ORR was not met, the combination of abemaciclib and paclitaxel in our study provided comparable benefits to previously reported findings of *CDK4/6* inhibitor monotherapy, without an apparent increase in toxicity. This highlights the potential importance of testing for bypass signaling pathway activation alongside *CDK4/6* activation. Despite some limitations, our findings provide insights that may guide appropriate treatment selection. This combination requires further investigation in a larger randomized trial involving a comparison to monotherapy.

ACKNOWLEDGEMENTS

The study drugs, abemaciclib (Verzenio®) and paclitaxel (Genexol® Inj), were kindly provided by Eli Lilly and Samyang Holdings Corp., respectively.

FUNDING

This study was supported by the Hanim Precision Medicine Center of Yonsei University Health System [grant number 6-2021-0219] and a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) [grant number 2021R1A2C1094530].

DISCLOSURE

HSK received grant/research support from MSD, Eli Lilly and Company, Ono Pharmaceutical Company, Medpacto Pharmaceutical, and Boryung Pharmaceutical Company outside the submitted work. SYR received grant/research support from MSD, Celltrion, Boehringer-Ingelheim, Eli Lilly and Company, Taiho, Bristol-Myers Squibb, ASLAN, and Incyte; and received consultation fees for Daiichi Sankyo, MSD, Eli Lilly, Bristol-Myers Squibb, and Eisail. SYR served on a speaker's bureau for Eli Lilly, Bristol-Myers Squibb, and MSD outside the submitted work. All other authors have declared no conflicts of interest.

DATA SHARING

The datasets used and/or analyzed during the study are available from the corresponding author upon reasonable request.

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