SUPPLEMENTARY MATERIAL

Supplementary Table S1. Characterization of CDK4/6 dependency according to signature

Signature	Known dependence	Known independence	Indeterminate dependence
PAM50	–	Basal-like	HER2-enriched, normal-like, luminal A/B
Lehmann	LAR	–	Basal-like 1/2, mesenchymal

CDK, cyclin-dependent kinase; HER2, human epidermal growth factor receptor 2; LAR, luminal androgen receptor; PAM50, Prediction Analysis of Microarray 50.

Supplementary Table S2. Subsequent anticancer therapy in the overall study population

	Group 1	**Group 2**	**Group 3**	**Groups 2 and 3**
Patients, *n*	34	33	35	68
Any subsequent anticancer therapy, *n* (%)	20 (58.8)	20 (60.6)	23 (65.7)	43 (63.2)
Median subsequent lines of systemic therapy, *n* (range)	1 (1–3)	1 (1–2)	2 (1–5)	1 (1–5)
1	11 (32.4)	11 (33.3)	8 (22.9)	19 (27.9)
2	3 (8.8)	4 (12.1%)	5 (14.3)	9 (13.2)
3	2 (5.9)	0	4 (11.4)	4 (5.9)
≥4	0	0	3 (8.6)	3 (4.4)
Pyrimidine analogues, *n* (%)				
Gemcitabine	6 (17.6)	5 (15.2)	11 (31.4)	16 (23.5)
Capecitabine	4 (11.8)	4 (12.1)	8 (22.9)	12 (17.6)
Platinum compound (carboplatin), *n* (%)	5 (14.7)	3 (9.1)	10 (28.6)	13 (19.1)
Taxanes, *n* (%)				
Paclitaxel	5 (14.7)	5 (15.1)	5 (14.3)	10 (14.7)
Docetaxel	2 (5.9)	1 (3.0)	1 (2.9)	2 (2.9)

Anthracyclines and related substances, *n* (%)				
Doxorubicin	0	1 (3.0)	3 (8.6)	4 (5.9)
Pegylated liposomal doxorubicin hydrochloride	0	0	4 (11.5)	4 (5.9)
Epirubicin	0	0	1 (2.9)	1 (1.5)
Vinca alkaloids and analogues (vinorelbine), *n* (%)	0	1 (3.0)	5 (14.3)	6 (8.9)
Nitrogen mustard analogues (cyclophosphamide), *n* (%)	0	1 (3.0)	1 (2.9)	2 (2.9)
Monoclonal antibodies, *n* (%)				
Pembrolizumab	4 (11.8)	3 (9.1)	2 (5.7)	5 (7.4)
Atezolizumab	0	1 (3.0)	2 (5.7)	3 (4.4)
Nivolumab	1 (2.9%)	0	1 (2.9)	1 (1.5)
Sacituzumab govitecan	0	1 (3.0)	1 (2.9)	2 (2.9)
Other antineoplastic agents, *n* (%)				
Eribulin	7 (20.6)	5 (15.2)	6 (17.1)	11 (16.2)
Olaparib	1 (2.9)	0	4 (11.4)	4 (5.9)

Alpelisib	0	0	1 (2.9)	1 (1.5)
Other	1 (2.9)	0	0	0
Poly-ADP-ribose polymerase inhibitor	0	0	1 (2.9)	1 (1.5)
Etinostat	0	0	1 (2.9)	1 (1.5)
Protein kinase inhibitors, *n* (%)				
Cediranib	0	0	1 (2.9)	1 (1.5)
Dabrafenib	0	1 (3.0)	0	1 (1.5)
Everolimus	0	1 (3.0)	0	1 (1.5)
Trametinib		1 (3.0)	0	1 (1.5)
Aromatase inhibitors, *n* (%)				
Anastrozole	0	0	1 (2.9)	1 (1.5)
Letrozole	0	1 (3.0)	0	1 (1.5)
Radiotherapy, *n* (%)	2 (5.9)	4 (12.1)	2 (5.7)	6 (8.8)
Investigational drug, *n* (%)	2 (5.9)	2 (6.1)	5 (14.3)	7 (10.3)
All other therapeutic products, *n* (%)	1 (2.9)	1 (3.0)	1 (2.9)	2 (2.9)

Group 1: chemotherapy on days 1 and 8; group 2: trilaciclib and chemotherapy on days 1 and 8; group 3: trilaciclib alone on days 1 and 8 and with chemotherapy on days 2 and 9.

Supplementary Table S3. Efficacy among patients with CDK4/6-dependent tumors

	Lehmann signature (LAR)			
	Group 1	**Group 2**	**Group 3**	**Groups 2 and 3**
Patients, *n*	9	10	9	19
ORR, *n* (%)	2 (22.2)	4 (40.0)	1 (11.1)	5 (26.3)
Median PFS, months (95% CI)	8.3 (4.8-NR)	11.6 (9.4-NR)	5.9 (2.7-NR)	9.4 (6.5-NR)
HR (95% CI)	–	0.39 (0.1-1.4)	1.3 (0.4-4.7)	0.65 (0.2-1.8)
Median OS, months (95% CI)	9.7 (7.5-NR)	NR (9.4-NR)	15.3 (7.5-NR)	15.3 (9.4-NR)
HR (95% CI)	–	0.18 (0.0-0.7)	0.49 (0.2-1.3)	0.32 (0.1-0.8)

Group 1: chemotherapy on days 1 and 8; group 2: trilaciclib and chemotherapy on days 1 and 8; group 3: trilaciclib alone on days 1 and 8 and with chemotherapy on days 2 and 9. HRs are for comparisons between group 2 and group 1, between group 3 and group 1, and between groups 2 and 3 combined and group 1.

CDK, cyclin-dependent kinase; CI, confidence interval; HR, hazard ratio; LAR, luminal androgen receptor; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

Supplementary Table S4. Efficacy among patients with CDK4/6-independent tumors

	PAM50 signature (basal-like)			
	Group 1	**Group 2**	**Group 3**	**Groups 2 and 3**
Patients, *n*	10	13	16	29
ORR, *n* (%)	3 (30.0)	6 (46.1)	9 (56.3)	15 (51.7)
Median PFS, months (95% CI)	5.4 (2.0-NR)	7.9 (6.1-NR)	10.9 (6.2-NR)	9.7 (6.2-14.0)
HR (95% CI)	–	0.78 (0.3-1.9)	0.79 (0.3-2.2)	0.75 (0.3-1.7)
Median OS, months (95% CI)	12.8 (6.3-NR)	19.6 (10.2-NR)	17.8 (12.9-NR)	19.6 (14.0-NR)
HR (95% CI)	–	0.56 (0.2-1.5)	0.64 (0.3-1.6)	0.60 (0.3-1.4)

Group 1: chemotherapy on days 1 and 8; group 2: trilaciclib and chemotherapy on days 1 and 8; group 3: trilaciclib alone on days 1 and 8 and with chemotherapy on days 2 and 9. HRs are for comparisons between group 2 and group 1, between group 3 and group 1, and between groups 2 and 3 combined and group 1.

CDK, cyclin-dependent kinase; CI, confidence interval; HR, hazard ratio; NR, not reached; ORR, objective response rate; OS, overall survival; PAM50, Prediction Analysis of Microarray 50; PFS, progression-free survival.

Supplementary Table S5. Efficacy among patients with variable/indeterminant CDK4/6 dependence

	PAM50 signature (non-basal)				Lehmann signature (basal-like 1/2, mesenchymal)			
	Group 1	**Group 2**	**Group 3**	**Groups 2 and 3**	**Group 1**	**Group 2**	**Group 3**	**Groups 2 and 3**
Patients, *n*	12	10	14	24	13	16	18	34
ORR, *n* (%)	4 (33.3)	4 (40.0)	5 (35.7)	9 (37.5)	5 (38.5)	9 (56.3)	10 (55.6)	19 (55.9)
Median PFS, months (95% CI)	8.3 (4.8-NR)	11.9 (8.8-NR)	7.3 (5.9-NR)	9.4 (7.3-NR)	5.7 (2.2-NR)	6.2 (4.3-NR)	10.9 (7.3-NR)	9.0 (6.2-15.5)
HR (95% CI)	–	0.42 (0.1-1.3)	0.71 (0.3-1.9)	0.57 (0.2-1.3)	–	0.80 (0.4-1.8)	0.55 (0.2-1.3)	0.65 (0.3-1.3)
Median OS, months (95% CI)	10.1 (7.5-18.8)	NR (9.4-NR)	22.3 (13.1-NR)	22.3 (13.0-NR)	16.0 (6.3-NR)	14.0 (10.2-NR)	22.3 (15.6-NR)	19.8 (14.0-NR)
HR (95% CI)	–	0.30 (0.1-0.8)	0.32 (0.1-0.8)	0.33 (0.2-0.7)	–	0.66 (0.3-1.5)	0.47 (0.2-1.1)	0.55 (0.3-1.1)

Group 1: chemotherapy on days 1 and 8; group 2: trilaciclib and chemotherapy on days 1 and 8; group 3: trilaciclib alone on days 1 and 8 and with chemotherapy on days 2 and 9. HRs are for comparisons between group 2 and group 1, between group 3 and group 1, and between groups 2 and 3 combined and group 1.

CDK, cyclin-dependent kinase; CI, confidence interval; HR, hazard ratio; NR, not reached; ORR, objective response rate; OS, overall survival; PAM50, Prediction Analysis of Microarray 50; PFS, progression-free survival.

Supplementary Table S6. Baseline characteristics in patients with PD-L1–positive and PD-L1–negative tumors

	Group 1 (*n* = 27)		Group 2 (*n* = 26)		Group 3 (*n* = 32)		Groups 2 and 3 (*n* = 58)		Total (*N* = 85)	
	PD-L1 positive	**PD-L1 negative**	**PD-L1 positive**	**PD-L1 negative**	**PD-L1 positive**	**PD-L1 negative**	**PD-L1 positive**	**PD-L1 negative**	**PD-L1 positive**	**PD-L1 negative**
Patients	17 (63.0)	10 (37.0)	16 (61.5)	10 (38.5)	16 (50.0)	16 (50.0)	32 (55.2)	26 (44.8)	49 (57.6)	36 (42.4)
Age										
≥65 years	4 (23.5)	3 (30.0)	4 (25.0)	5 (50.0)	6 (37.5)	3 (18.8)	10 (31.2)	8 (30.7)	14 (28.6)	11 (30.6)
<65 years	13 (76.5)	7 (70.0)	12 (75.0)	5 (50.0)	10 (62.5)	13 (81.2)	22 (68.8)	18 (69.2)	35 (71.4)	25 (69.4)
Race										
White	12 (70.6)	9 (90.0)	13 (81.2)	5 (50.0)	11 (68.8)	14 (87.5)	24 (75.0)	19 (73.1)	36 (73.5)	28 (77.8)
Non-White	5 (29.4)	1 (10.0)	3 (18.8)	5 (50.0)	5 (31.2)	2 (12.4)	8 (25.0)	7 (26.9)	13 (26.5)	8 (22.2)
Country										
USA	16 (94.1)	7 (70.0)	12 (75.0)	10 (100)	13 (81.2)	13 (81.2)	25 (78.1)	23 (88.5)	41 (83.7)	30 (83.3)
Non-USA	1 (5.9)	3 (30.0)	4 (24.9)	0	3 (18.6)	3 (18.7)	7 (21.8)	3 (11.5)	8 (16.3)	6 (16.7)
ECOG PS										
0	8 (47.1)	3 (30.0)	10 (62.5)	3 (30.0)	11 (68.8)	10 (62.5)	21 (65.6)	13 (50.0)	29 (59.2)	16 (44.4)
1	9 (52.9)	7 (70.0)	6 (37.5)	7 (70.0)	5 (31.2)	6 (37.5)	11 (34.4)	13 (50.0)	20 (40.8)	20 (55.6)

TNBC histological classification										
Acquired	2 (11.8)	2 (20.0)	2 (12.5)	4 (40.0)	2 (12.5)	8 (50.0)	4 (12.5)	12 (46.2)	6 (12.2)	14 (38.9)
Pure	15 (88.2)	6 (60.0)	13 (81.2)	6 (60.0)	14 (87.5)	6 (37.5)	27 (84.4)	12 (46.2)	42 (85.7)	18 (50.0)
Unknown	–	2 (20.0)	1 (6.2)	–	–	2 (12.5)	1 (3.1)	2 (7.7)	1 (2.0)	4 (11.1)
Liver involvement										
Yes	4 (23.5)	3 (30.0)	5 (31.2)	3 (30.0)	4 (25.0)	5 (31.2)	9 (28.1)	8 (30.8)	13 (26.5)	11 (30.6)
No	13 (76.5)	7 (70.0)	11 (86.8)	7 (70.0)	12 (75.0)	11 (68.8)	23 (71.9)	18 (69.2)	36 (73.5)	25 (69.4)
Prior lines of chemotherapy										
1 or 2	8 (47.1)	2 (20.0)	6 (37.5)	2 (20.0)	10 (62.5)	4 (25.0)	16 (50.0)	6 (23.1)	24 (49.0)	8 (22.2)
0	9 (52.9)	8 (80.0)	10 (62.5)	8 (80.0)	6 (37.5)	12 (75.0)	16 (50.0)	20 (76.9)	25 (51.0)	28 (77.8)

Values are presented as *n* (%).

ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed death ligand-1; TNBC, triple-negative breast cancer.

Supplementary Table S7. Tumor response, PFS, and OS according to PD-L1 status

	PD-L1 positive				PD-L1 negative			
	Group 1	**Group 2**	**Group 3**	**Groups 2 and 3**	**Group 1**	**Group 2**	**Group 3**	**Groups 2 and 3**
Patients analyzed, *n*	17	16	16	32	10	10	16	26
ORR, *n* (%)	4 (23.5)	8 (50.0)	7 (43.8)	15 (46.9)	3 (30.0)	4 (40.0)	4 (25.0)	8 (30.7)
Median PFS, months (95% CI)	5.4 (3.3-NR)	7.9 (6.1-NR)	10.9 (6.2-NR)	9.7 (6.2-15.5)	9.2 (8.3-NR)	11.9 (8.8-NR)	9.0 (6.4-NR)	9.4 (6.5-14.6)
HR (95% CI)	–	0.74 (0.3-1.7)	0.41 (0.2-1.1)	0.57 (0.3-1.2)	–	0.60 (0.2-1.9)	1.47 (0.5-4.3)	0.97 (0.4-2.5)
Median OS, months (95% CI)	10.5 (6.3-18.8)	20.1 (10.2-NR)	32.7 (15.3-NR)	32.7 (17.7-NR)	13.9 (12.6-NR)	NR (9.4-NR)	17.8 (12.9-NR)	17.8 (13.1-NR)
HR (95% CI)	–	0.38 (0.2-1.0)	0.30 (0.1-0.8)	0.34 (0.2-0.7)	–	0.35 (0.1-1.2)	0.55 (0.2-1.4)	0.48 (0.2-1.2)

Group 1: chemotherapy on days 1 and 8; group 2: trilaciclib prior to chemotherapy on days 1 and 8; group 3: trilaciclib alone on days 1 and 8 and prior to chemotherapy on days 2 and 9. HRs are for comparisons between group 2 and group 1, between group 3 and group 1, and between groups 2 and 3 combined and group 1.

CI, confidence interval; HR, hazard ratio; NR, not reached; ORR, objective response rate; OS, overall survival; PD-L1, programmed death ligand-1; PFS, progression-free survival.

Supplementary Table S8. Tumor response, PFS, and OS according to immune subtypes

Subtype	**High/class 2**				**Low/not class 2**			
	Group 1	**Group 2**	**Group 3**	**Groups 2 and 3**	**Group 1**	**Group 2**	**Group 3**	**Groups 2 and 3**
Ayer's IFNγ signature, *n*	13	11	12	23	9	15	15	30
ORR, *n* (%)	5 (38.5)	7 (63.6)	6 (50.0)	13 (56.5)	2 (22.2)	5 (33.3)	6 (40.0)	11 (36.7)
Median PFS, months (95% CI)	5.7 (5.4-NR)	13.0 (11.3-NR)	9.0 (6.5-NR)	11.3 (7.3-NR)	8.3 (2.0-NR)	13.9 (3.9-NR)	7.9 (6.1-NR)	8.8 (6.1-14.6)
HR (95% CI)	–	0.40 (0.1-1.2)	0.59 (0.2-1.5)	0.49 (0.2-1.1)	–	0.85 (0.3-2.3)	0.90 (0.3-2.7)	0.87 (0.3-2.2)
Median OS, months (95% CI)	12.8 (9.7-NR)	20.1 (7.1-NR)	22.3 (17.8-NR)	22.3 (15.3-NR)	8.3 (6.3-NR)	15.3 (8.7-NR)	19.6 (10.2-NR)	15.6 (12.9-NR)
HR (95% CI)	–	0.44 (0.2-1.2)	0.35 (0.1-0.9)	0.40 (0.2-0.9)	–	0.30 (0.1-0.9)	0.41 (0.2-1.1)	0.37 (0.2-0.9)
Ayer's expanded IFNγ signature, *n*	13	10	14	24	9	13	16	29
ORR, *n* (%)	5 (38.5)	6 (60.0)	6 (42.9)	12 (50.0)	2 (22.2)	5 (38.5)	7 (43.8)	12 (41.4)

Median PFS, months (95% CI)	5.7 (4.8-NR)	11.3 (8.8-NR)	9.0 (6.2-NR)	9.7 (7.3-20.1)	8.3 (2.0-NR)	13.9 (5.9-NR)	7.9 (6.1-NR)	9.4 (6.1-15.5)
HR (95% CI)	–	0.39 (0.1-1.2)	0.56 (0.2-1.5)	0.47 (0.2-1.1)	–	1.0 (0.4-2.7)	1.2 (0.4-3.6)	1.1 (0.4-2.7)
Median OS, months (95% CI)	12.8 (9.7-NR)	NR (7.1-NR)	19.8 (15.3-NR)	20.1 (15.3-NR)	9.1 (6.3-NR)	17.7 (12.9-NR)	14.0 (10.2-NR)	15.6 (12.9-NR)
HR (95% CI)	–	0.38 (0.1-1.0)	0.44 (0.2-1.1)	0.41 (0.2-0.9)	–	0.40 (0.1-1.1)	0.38 (0.1-1.0)	0.40 (0.2-0.9)
Thorsson six-class immune signature, *n*	10	17	18	35	12	9	9	18
ORR, *n* (%)	3 (30.0)	9 (52.9)	8 (44.4)	17 (48.6)	4 (33.3)	3 (33.3)	4 (44.4)	7 (38.9)
Median PFS, months (95% CI)	9.2 (5.4-NR)	8.8 (6.2-NR)	10.9 (6.5-NR)	10.9 (6.5-14.0)	5.4 (3.3-NR)	7.3 (1.2-NR)	9.7 (2.1-NR)	9.4 (5.9-15.6)
HR (95% CI)	–	0.75 (0.3-2.0)	0.65 (0.2-1.7)	0.69 (0.3-1.7)	–	0.63 (0.2-1.8)	0.99 (0.4-2.7)	0.76 (0.3-1.8)
Median OS, months (95% CI)	12.8 (5.8-NR)	NR (13.0-NR)	22.3 (15.3-NR)	32.7 (15.3-NR)	10.2 (7.5-18.8)	13.1 (8.7-NR)	14.8 (9.4-NR)	13.1 (9.4-NR)
HR (95% CI)	–	0.47 (0.2-1.2)	0.45 (0.2-1.1)	0.46 (0.2-1.0)	–	0.42 (0.1-1.2)	0.52 (0.2-1.3)	0.49 (0.2-1.0)

Group 1: chemotherapy on days 1 and 8; group 2: trilaciclib prior to chemotherapy on days 1 and 8; group 3: trilaciclib alone on days 1 and 8 and prior to chemotherapy on days 2 and 9. HRs are for comparisons between group 2 and group 1, between group 3 and group 1, and between groups 2 and 3 combined and group 1. Class 2 was defined as IFNγ dominant. Not adjusted for multiplicity.

CI, confidence interval; HR, hazard ratio; IFNγ, interferon gamma; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.