Table 1. Clinical characteristics of 347 patients with CDK4/6, CDKN2A/B aberrations (univariate analysis)

Patient characteristics (N=347)	**Aberrant CDK4/6, DKN2A/B N=79 (%)**	**Normal CDK4/6, CDKN2A/B N=268 (%)**	**p-value***
Gender			**0.026**
Women (N=196)	36 (18.4%)	160 (81.6%)	
Men (N=151)	43 (28.5%)	108 (71.5%)	
Age at diagnosis			0.13
Age ≥ 50 years (N=212)	54 (25.5%)	158 (74.5%)	
Age <50 years (N=135)	25 (18.5%)	110 (81.5%)	
Types of cancer diagnosis§			
Breast (N=74)	7 (9.5%)	67 (90.5%)	**0.0021**
Glioblastoma (N=26)	21 (80.8%)	5 (19.2%)	**< 0.0001**
Colorectal (N=26)	0 (0%)	26 (100%)	**0.004**
Lung (N=23)	6 (26.1%)	17 (73.9%)	0.69
Melanoma (N=23)	8 (34.8%)	15 (65.2%)	0.16
Appendix (N=20)	1 (5.0%)	19 (95.0%)	0.27
Site of metastasis (N=280) ¶	**Aberrant CDK4/6, DKN2A/B N=50 (%)**	**Normal CDK4/6, CDKN2A/B N=230 (%)**	
Lymph node metastasis			0.73
Yes (N=145)	27 (18.6%)	118 (81.4%)	
No (N=135)	23 (17.0%)	112 (83.0%)	
Liver metastasis			0.74
Yes (N=101)	17 (16.8%)	84 (83.2%)	
No (N=179)	33 (18.4%)	146 (81.6%)	
Bone metastasis			0.36
Yes (N=82)	12 (14.6%)	70 (85.4%)	
No (N=198)	38 (19.2%)	160 (80.8%)	
Lung metastasis			0.23
Yes (N=81)	11 (13.6%)	70 (86.4%)	
No (N=199)	39 (19.6%)	160 (80.4%)	
Omentum/Peritoneum metastasis			0.92
Yes (N=49)	9 (18.4%)	40 (81.6%)	
No (N=231)	41(17.8%)	190 (82.2%)	
Brain metastasis			0.16
Yes (N=34)	9 (26.5%)	25 (73.5%)	
No (N=246)	41(16.7%)	205 (83.3%)	
Soft tissue metastasis			0.21
Yes (N=31)	3 (9.7%)	28 (90.3%)	
No (N=249)	47 (18.9%)	202 (81.1%)	
Adrenal metastasis			0.51
Yes (N=12)	3 (25.0%)	9 (75.0%)	
No (N=268)	47 (17.5%)	221 (82.5%)	
Site of biopsies			0.83
Primary (N=125)	23 (18.4%)	102 (81.6%)	
Metastases (N=155)	27 (17.4%)	128 (82.6%)	

* p-values are from Fisher's exact test.

§ Included characteristics with N ≥ 20 of primary cancer diagnosis. See supplemental table 1 for complete list of characteristics with N <20.

¶ Excluded patients with hematological malignancy (N=21) and CNS tumors (N=46). Reported here with site of metastasis with N ≥ 10.

Table 2. CDK4/6 or CDKN2A/B abnormality and types of co-existing genetic aberrations (univariate analysis)

Patient characteristics (N=347)	**Aberrant CDK4/6, CDKN2A/B N=79 (%)**	**Normal CDK4/6, CDKN2A/B N=268 (%)**	**p-value***
Types of co-existing genetic aberrations§			
TP53			0.28
Wild-type (N=194)	40 (20.6%)	154 (79.4%)	
Aberrant (N=153)	39 (25.5%)	114 (74.5%)	
KRAS			**0.02**
Wild-type (N=292)	73 (25.0%)	219 (75.0%)	
Aberrant (N=55)	6 (10.9%)	49 (89.1%)	
FGFR/FGF			0.11
Wild-type (N=296)	63 (21.3%)	233 (78.7%)	
Aberrant (N=51)	16 (31.4%)	35 (68.6%)	
PIK3CA			**0.01**
Wild-type (N=307)	76 (24.8%)	231 (75.2%)	
Aberrant (N=40)	3 (7.5%)	37 (92.5%)	
PTEN			0.96
Wild-type (N=308)	70 (22.7%)	238 (77.3%)	
Aberrant (N=39)	9 (23.1%)	30 (76.9%)	
MYC			0.24
Wild-type (N=311)	68 (21.9%)	243 (78.1%)	
Aberrant (N=36)	11 (30.6%)	25 (69.4%)	
EGFR			**< 0.0001**
Wild-type (N=321)	59 (18.4%)	262 (81.6%)	
Aberrant (N=26)	20 (76.9%)	6 (23.1%)	
CCND1			0.52
Wild-type (N=322)	72 (22.4%)	250 (77.6%)	
Aberrant (N=25)	7 (28.0%)	18 (72.0%)	
APC			**0.03**
Wild-type (N=324)	78 (24.1%)	246 (75.9%)	
Aberrant (N=23)	1 (4.4%)	22 (95.7%)	
MCL1			1.00
Wild-type (N=325)	74 (22.8%)	251 (77.2%)	
Aberrant (N=22)	5 (22.7%)	17 (77.3%)	
NF1			1.00
Wild-type (N=325)	74 (22.8%)	251 (77.2%)	
Aberrant (N=22)	5 (22.7%)	17 (77.3%)	
ARID1A			**0.06**
Wild-type (N=327)	71 (21.7%)	256 (78.3%)	
Aberrant (N=20)	8 (40.0%)	12 (60.0%)	
BRCA2			0.39
Wild-type (N=327)	76 (23.2%)	251 (77.2%)	
Aberrant (N=20)	3 (15.0%)	17 (85.0%)	

* p-values are from Fisher's exact test.
§ Included characteristics with N ≥ 20 of genetic aberration. See supplemental table 1 for complete list of characteristics with N <20.

Table 3. Multivariate analysis of patient characteristics (N=347) associated with CDK4/6, CDKN2A/B aberrations

Characteristics	Odds ratio (95% CI)	p-value*
Women	0.92 (0.48-1.76)	0.80
Breast	0.39 (0.15-1.02)	0.057
Glioblastoma	11.2 (3.7-34.5)	**< 0.0001**
Colorectal	0.28 (0.03-2.53)	0.26
KRAS aberrant	0.59 (0.22-1.57)	0.29
PIK3CA aberrant	0.56 (0.16-1.96)	0.36
EGFR aberrant	11.9 (3.92-35.7)	**< 0.0001**
APC aberrant	0.19 (0.02-1.83)	0.15
ARID1A aberrant	3.98 (1.36-11.8)	**0.01**

Characteristics selected from table 1 and 2 with p-value <0.1.
*p-values are from multivariate logistic regression analysis.
P <0.05 was considered to be statistically significant.

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Table 4. Clinical outcomes of patients with CDK4/6, CDKN2A/B aberrations (univariate analysis)

Clinical outcomes	Aberrant CDK4/6, CDKN2A/B Month (range)	Normal CDK4/6, CDKN2A/B Month (range)	p-value*	Comment
Median time from diagnosis to metastasis (N=280)	17.0 (0-256.7) N=50	24.2 (0-415.1) N=230	0.20	(a)
PFS for first line therapy				
Median PFS for all first line therapy (N=189)	3.3 (0.6-39.0) N=41	5.0 (0.5-61.0) N=148	0.32	(b)
Subgroup analysis of median PFS for first line therapy				
Platinum-containing regimen (N=62)	3.5 (0.6-11.0) N=15	5.0 (1.0-34.0) N=47	0.13	(c)
5-FU or capecitabine-containing regimen (N=50)	5.0 (0.6-11.0) N=11	5.0 (0.8-21.0) N=39	0.3	(d)
Bevacizumab-containing regimen (N=33)	6.5 (2.0-39.0) N=6	6.5 (1.0-34.0) N=27	0.43	
Taxane-containing regimen (N=32)	3.0 (0.6-39.0) N=7	9.1 (1.0-38.7) N=25	0.88	(e)

* p-values are from log-rank test.
(a) Excluded patients with hematological malignancy (N=21) and CNS tumors (N=46).
(b) First line therapy referred to first line after metastatic or recurrent disease (N = 189). Excluded patients with hematological malignancy (N=21). Neoadjuvant/adjuvant therapy was not included (N=56). Chemotherapy was not initiated on 33 patients and 48 patients were not assessable for accurate PFS for first line therapy.
(c) Platinum-containing regimen (N=62): Cisplatin (N=20), carboplatin (N=21) and oxaliplatin (N=21).
(d) 5-FU or capecitabine-containing regimen (N=50): 5-FU (N=30) and capecitabine (N=20)
(e) Taxane-containing regimen (N=32): Paclitaxel (N=22), docetaxel (N=5) and abraxane (N=5).

Table 5. Univariate and multivariate Cox's regression models predicting duration of overall survival in 347 patients with malignancies

Characteristics¶ (N=347)	**Hazard Ratio**	**95% CI**	**p-value***
Gender and age			
Women (N=196)	0.65	0.38-1.04	**0.08**
Age ≥ 50 years (N=212)	1.21	0.77-1.97	0.41
Types of cancer			
Breast (N=74)	0.64	0.39-1.08	0.11
Glioblastoma (N=26)	3.01	1.73-26.7	**0.006**
Colorectal (N=26)	1.73	0.71-5.93	0.19
Lung (N=23)	1.16	0.40-3.46	0.77
Melanoma (N=23)	0.55	0.25-1.48	0.29
Appendix (N=20)	0.89	0.30-2.67	0.84
Types of genetic aberration			
TP53 aberrant (N=153)	2.00	1.27-3.31	**0.004**
CDK4/6, CDKN2A/B aberrant (N=79)	2.09	1.35-4.70	**0.004**
KRAS aberrant (N=55)	1.24	0.63-2.53	0.51
FGFR/FGF aberrant (N=51)	0.67	0.39-1.26	0.24
PIK3CA aberrant (N=40)	0.76	0.39-1.57	0.49
PTEN aberrant (N=39)	4.02	5.02-31.9	**<0.0001**
MYC aberrant (N=36)	1.45	0.71-3.33	0.27
EGFR aberrant (N=26)	2.23	1.07-9.71	**0.04**
CCND1 aberrant (N=25)	0.48	0.27-1.21	0.15
APC (N=23)	1.75	0.65-6.69	0.22
MCL1 aberrant (N=22)	0.49	0.25-1.35	0.21
NF1 aberrant (N=22)	0.74	0.28-2.10	0.61
ARID1A aberrant (N=20)	0.61	0.26-1.65	0.38
BRCA2 aberrant (N=20)	1.09	0.38-3.12	0.87
Multivariate Cox's regression model (N=347)			
Women	0.60	0.35-1.02	0.06
Glioblastoma	1.43	0.55-3.72	0.46
TP53 aberrant	1.92	1.17-3.14	**0.01**
CDK4/6, CDKN2A/B aberrant	1.67	0.92-3.04	0.09
PTEN aberrant	4.83	2.63-8.87	**<0.0001**
EGFR aberrant	1.31	0.54-3.16	0.55

¶ Included characteristics with N ≥ 20.

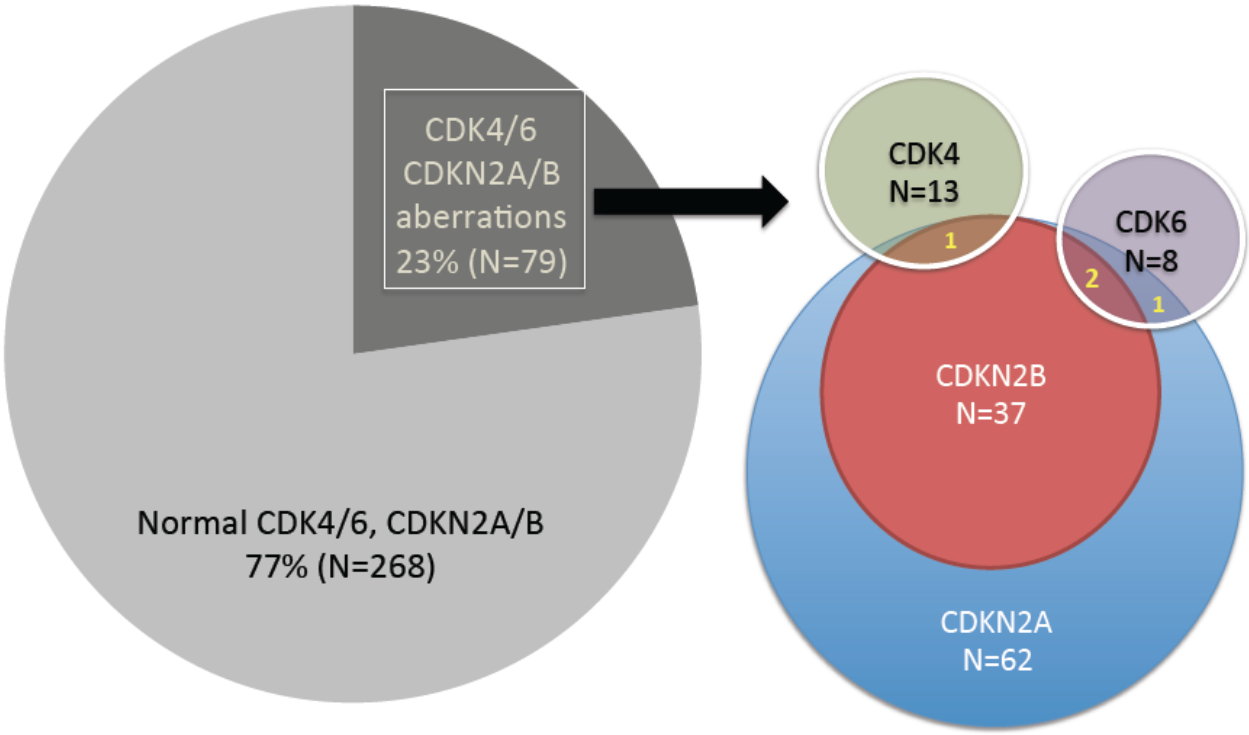
* p-values (univariate) and hazard ratios with 95% CI are from log-rank test or multivariate Cox's regression model as appropriate. p <0.1 from univariate analysis were included for multivariate analysis.

FIGURE LEGEND

Figure 1. Frequency of CDK-associated genetic aberrations in 347 patients with diverse malignancies

Of 347 patients with diverse malignancies, 23% (N=79) had an aberration in either CDK4 (N = 13 [3.7% of 347 patients]), CDK6 (N = 8 [2.3%]), CDKN2A (N = 62 [17.9%]) and/or CDKN2B (N = 37 [10.7%]). All cases with CDKN2B aberrations (N=37) also had aberrant CDKN2A (but the opposite was not true). One case of CDK4 amplification had a co-existing CDKN2A/B aberration. CDK6 amplification occasionally co-existed with CDKN2A/B aberration (N=2) or a CDKN2A aberration (N=1).

Figure 1.
Frequency of CDK-associated genetic aberrations in 347 patients with advanced Malignancies



CDKN2A (N = 62): Loss = 48; point mutation = 13; splicing mutation = 1
CDKN2B (N = 37): All due to loss
CDK4/6 (N = 21): All due to amplification

Supplemental Table 1. Univariate analysis of CDK4/6 and CDKN2A/B aberrations among diagnoses or other variables with < 20 of cases

Characteristics N=347	Aberrant CDK4/6, CDKN2A/B N=79 (%)	Normal CDK4/6, CDKN2A/B N=268 (%)	p-value*
Types of cancer diagnosis			
Head and neck (N=18)	6 (33.33%)	12 (66.67%)	0.27
Ovarian (N=10)	2 (20.00%)	8 (80.00%)	0.83
Soft tissue/Sarcoma[¥] (N=9)	3 (33.33%)	6 (66.67%)	0.44
Other[€] (N=9)	2 (22.22%)	7 (77.78%)	0.97
Pancreas (N=8)	2 (25.00%)	6 (75.00%)	0.88
Astrocytoma (N=7)	2 (28.57%)	5 (71.43%)	0.71
Cholangiocarcinoma (N=7)	1 (14.29%)	6 (85.71%)	0.59
Thyroid (N=7)	1 (14.29%)	6 (85.71%)	0.59
Gastric (N=6)	3 (50.00%)	3 (50.00%)	0.11
Chronic lymphocytic leukemia (N=6)	1 (16.67%)	5 (83.33%)	0.72
Oligodendroglioma (N=5)	1 (20.00%)	4 (80.00%)	0.88
Acute myeloid leukemia (N=5)	0 (0.00%)	5 (100.00%)	0.22
Carcinoma of unknown primary (N=5)	3 (60.00%)	2 (40.00%)	0.05
Neuroendocrine (N=5)	0 (0.00%)	5 (100.00%)	0.22
Meningioma[¶] (N=4)	2 (50.00%)	2 (50.00%)	0.19
Lymphoma (N=4)	1 (25.00%)	3 (75.00%)	0.91
Myelodysplastic syndrome (N=4)	0 (0.00%)	4(100.00%)	0.27
Esophageal (N=4)	2 (50.00%)	2 (50.00%)	0.19
Squamous cell carcinoma of skin (N=4)	2 (50.00%)	2 (50.00%)	0.19
Anal (N=3)	0 (0.00%)	3 (100.00%)	0.34
Cervix (N=3)	0 (0.00%)	3 (100.00%)	0.34
Small intestine (N=3)	0 (0.00%)	3 (100.00%)	0.34
Multiple myeloma (N=2)	0 (0.00%)	2 (100.00%)	0.44
Adenoid cystic carcinoma(N=2)	0 (0.00%)	2 (100.00%)	0.44
Merkel cell carcinoma (N=2)	0 (0.00%)	2 (100.00%)	0.44
Renal cell carcinoma (N=2)	1 (50.00%)	1 (50.00%)	0.36
Uterine leiomyosarcoma (N=2)	0 (0.00%)	2 (100.00%)	0.44
Oligoastrocytoma (N=2)	1 (50.00%)	1 (50.00%)	0.36
Mixed glioma (N=1)	0 (0.00%)	1 (100.00%)	0.59
Pituitary cancer (N=1)	0 (0.00%)	1 (100.00%)	0.59
Basal cell carcinoma (N=1)	0 (0.00%)	1 (100.00%)	0.59
Bladder cancer (N=1)	0 (0.00%)	1 (100.00%)	0.59
Carcinoid (N=1)	0 (0.00%)	1 (100.00%)	0.59
Endometrium (N=1)	0 (0.00%)	1 (100.00%)	0.59
Vulvar cancer (N=1)	0 (0.00%)	1 (100.00%)	0.59
Types of co-existing genetic aberration[§]			
ZNF (N=19)	3 (15.79%)	16 (84.21%)	0.62
SMAD4 (N=18)	4 (22.22%)	14 (77.78%)	0.97

* p-values are from Fisher's exact test.

§ Genetic aberrations with > 5% of frequencies are reported.

Supplemental Table 1. Univariate analysis of CDK4/6 and CDKN2A/B aberrations among diagnoses or other variables with < 20 of cases

¥ Soft tissue/ Sarcoma (N=9):
Ewing sarcoma (N=1), epithelioid sarcoma (N=1), leiomyosarcoma (N=1), myoepithelial carcinoma of soft tissue (N=1), sarcomatoid neoplasm (N=1), pleomorphic rhabdomyosarcoma (N=1), rhabdomyosarcoma of soft tissue (N=1), soft tissue liposarcoma (N=2)

€ Other (N=9):
Anaplastic ependymoma (N=1), malignant peripheral nerve sheath tumor (N=1), malignant fibrous histiocytoma (N=1), ampulla of vater carcinoma (N=1), thymic carcinoma (N=2), metastatic paraganglioma (N=1), peritoneum serous carcinoma (N=1), metastatic hidradenoma (N=1)

¶ Meningioma (N=4): WHO Grade 2 (N=2), WHO Grade 3 (N=2)

Supplemental Table 2. Characteristics of patients with co-existing EGFR and CDK4/6, CDKN2A/B aberration

Patient ID	Diagnosis	Types of aberrant EGFR	Types of aberrant CDK4/6, CDKN2A/B
4	Breast Cancer	EGFR amplification	CDKN2A/B loss
12	Tongue Cancer	EGFR amplification	CDKN2A R58*
164	Non-small cell carcinoma of lung	EGFR G719A, L861Q	CDK4 amplification
209	Astrocytoma and angiocentric glioma	EGFR amplification	CDKN2A loss
220	Astrocytoma	EGFR amplification, EGFRvIII	CDKN2A/B loss
232	Glioblastoma	EGFR amplification, EGFRvIII	CDKN2A/B loss
243	Glioblastoma	EGFR A289V, EGFR amplification	CDK4 amplification
257	Glioblastoma	EGFR amplification	CDK6 amplification
259	Glioblastoma	EGFR amplification, EGFRvIII	CDKN2A/B loss
312	Esophageal cancer	EGFR amplification – equivocal	CDK6 amplification, CDKN2A L16_A17insAT
317	Glioblastoma	EGFR amplification	CDKN2A/B loss
337	Oligoastrocytoma	EGFR amplification	CDKN2A/B loss
339	Glioblastoma	EGFR amplification, EGFRvIII	CDKN2A/B loss
404	Esophageal cancer	EGFR amplification	CDK6 amplification
459	Appendiceal cancer	EGFR amplification	CDK6 amplification
463	Oligodendroglioma	EGFR amplification	CDKN2A loss
465	Lung adenocarcinoma	EGFR E746_A750del	CDK6 amplification
477	Glioblastoma	EGFR amplification	CDKN2A/B loss
489	Lung adenocarcinoma	EGFR E746_A750del	CDKN2A/B loss
495	Glioblastoma	EGFR amplification, EGFRvIII	CDKN2A/B loss

Supplemental Table 3. Characteristics of patients with co-existing ARID1A and CDK4/6, CDKN2A/B aberration

Patient ID	Diagnosis	Types of aberrant ARID1A	Types of aberrant CDK4/6, CDKN2A/B
49	Gastric cancer	ARID1A P2139fs*62	CDKN2A loss
146	Thymic carcinoma	ARID1A R1335*	CDKN2A/B loss
167	Melanoma	ARID1A Y1377*	CDKN2A R80*
183	Glioblastoma	ARID1A L2089P	CDKN2A/B loss
197	Breast cancer	ARID1A 253fs*111	CDK4 amplification
221	Glioblastoma	ARID1A A343_A348>A	CDK4 amplification
253	Cholangiocarcinoma	ARID1A A1136fs*50	CDK4 amplification
469	Carcinoma of unknown primary	ARID1A S11fs*91	CDKN2A V51I

SUPPLEMENTAL FIGURE LEGEND

Supplemental Figure1. **Cyclin D- CDK-Rb pathway: A key pathway in G1 cell cycle progression**. CyclinD1 (CCND1) complexes with CDK4/6 leading to phosphorylation of Rb and disassociation of cellular transcription factor E2F from Rb. The latter allows E2F to promote cell cycle progression through G1. CDKN2A/B (CDKN2A = p16; CDKN2B = p15) inhibits CDK4/6, blocking the kinase active site and preventing association with the cyclins. This leads to activation of Rb (active Rb is hypophosphorylated), which in turn negatively regulates E2F and prevents cell cycle entry (causing cell cycle arrest).

CDK4/6 amplification or CDKN2A/B loss would therefore be associated with enhanced cell cycle progression. Inhibitors of CDK4/6 would attenuate the latter effect and may be useful in cancer therapy. However, when Rb is mutated, it can lead to functional inactivation by phosphorylation. Subsequently, E2F transcription factors are liberated and induce cell cycle progression. Hence, Rb mutations would confer resistance to CDK4/6 inhibitors.

Supplemental Figure1. Cyclin D- CDK-Rb pathway: A key pathway in G1 cell cycle progression