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Cyclin Pathway Genomic Alterations Across 190,247 Solid Tumors: Leveraging Large-Scale Data to Inform Therapeutic Directions

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Key Words. Cell cycle • CDK4 • CDK6 • Precision oncology • Molecular genetics • Cancer genome • Targeted therapy

Abstract

Background. We describe the landscape of cyclin and interactive gene pathway alterations in 190,247 solid tumors.

Methods. Using comprehensive genomic profiling (315 genes, >500× coverage), samples were analyzed for alterations in activating/sensitizing cyclin genes (*CDK4* amplification, *CDK6* amplification, *CCND1*, *CCND2*, *CCND3*, *CDKN2B* [loss], *CDKN2A* [loss], *SMARCB1*), hormone genes (estrogen receptor 1 [*ESR1*], androgen receptor [*AR*]), and co-alterations in genes leading to cyclin inhibitor therapeutic resistance (*RB1* and *CCNE1*).

Results. Alterations in at least one cyclin activating/sensitizing gene occurred in 24% of malignancies. Tumors that frequently harbored at least one cyclin alteration were brain gliomas (47.1%), esophageal (40.3%) and bladder cancer (37.9%), and mesotheliomas (37.9%). The most frequent alterations included *CDKN2A* (13.9%) and *CDKN2B* loss (12.5%). Examples of unique patterns of alterations included *CCND1* amplification in breast cancer (17.3%); *CDK4* alterations in sarcomas (12%); *CCND2* in testicular cancer (23.4%), and *SMARCB1* mutations in kidney cancer (3% overall, 90% in malignant rhabdoid tumors). Alterations in resistance genes *RB1* and *CCNE1* affected 7.2% and 3.6% of samples. Co-occurrence analysis demonstrated a lower likelihood of concomitant versus isolated alterations in cyclin activating/sensitizing and resistance genes (odds ratio [OR], 0.35; $p < .001$), except in colorectal, cervical, and small intestine cancers. *AR* and cyclin activating/sensitizing alterations in prostate cancer co-occurred more frequently (vs. *AR* alterations and wild-type cyclin activating/sensitizing alterations) (OR, 1.79; $p < .001$) as did *ESR1* and cyclin activating/sensitizing alterations in breast (OR, 1.62; $p < .001$) and cervical cancer (OR, 4.08; $p = .04$) (vs. *ESR1* and cyclin wild-type activating/sensitizing alterations).

Conclusion. Cyclin pathway alterations vary according to tumor type/histology, informing opportunities for targeted therapy, including for rare cancers. ***The Oncologist*** 2021;26:e78–e89

Implications for Practice: Cyclin pathway genomic abnormalities are frequent in human solid tumors, with substantial variation according to tumor site and histology. Opportunities for targeted therapy emerge with comprehensive profiling of this pathway.

Introduction

Proliferation of normal cells is tightly controlled during the cell cycle. Cyclin-dependent kinases (CDKs), upon ligation to cyclin proteins, play a major role in these processes. At the transition of G1 to S phase of mitosis, cyclin D interacts with CDK4 and CDK6, and cyclin E interacts with CDK2 to form complexes that phosphorylate and inactivate retinoblastoma proteins (Rb) [1]. Phosphorylated Rb1 releases the early region 2 binding factor (E2F), which constitutes a complex family of transcriptional regulators that ultimately promote cell proliferation (Fig. 1) [2]. Other cyclins, including cyclins A and B and their associated CDKs, also exert regulatory functions during the subsequent steps of cell cycle regulation [3].

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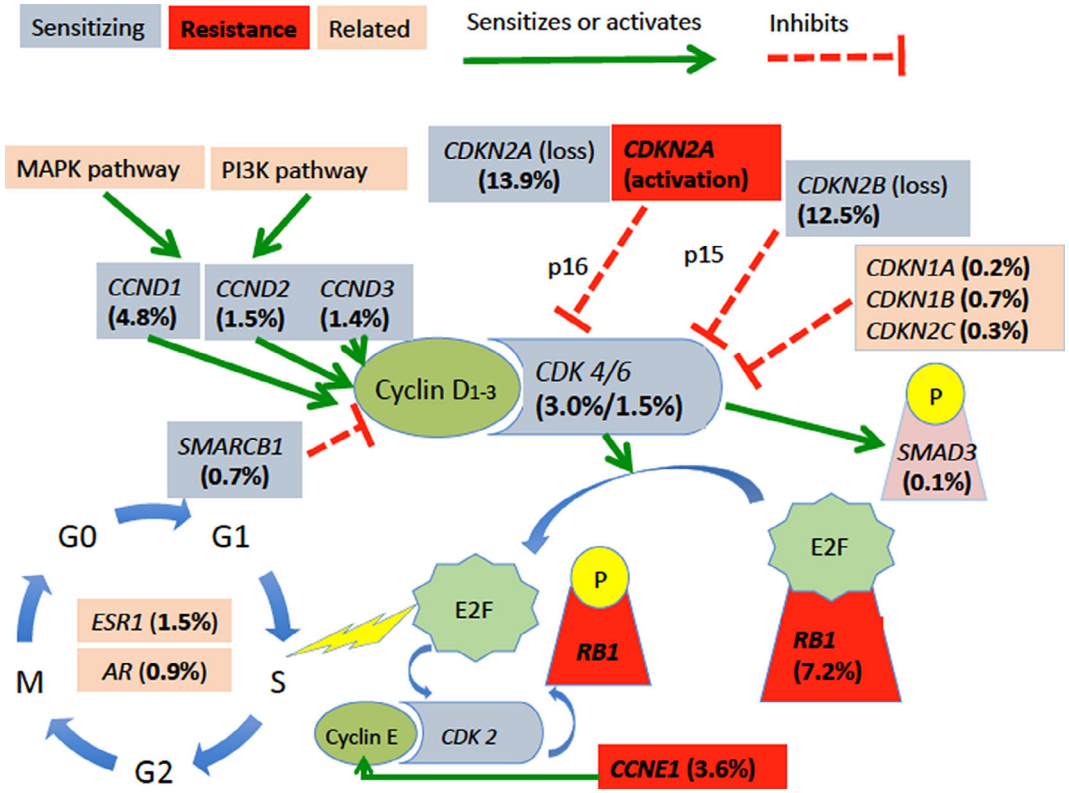

Figure 1. Genomic alterations in the cyclin pathway in patients with cancer. Schematic representation of genes that are part of the cyclin pathway, including their relationship with the mitotic cycle and transition from G1 to S phase. Genes that are shaded in light gray may suffer genomic alterations that can lead to cyclin pathway upregulation. Alterations in genes that could lead to resistance to cyclin inhibitors (supplemental online Table 1) are shaded in red. Numbers in brackets are the frequencies of genomic alterations in each gene detected in the current study in the overall population of 190,247 solid tumors. Dotted arrows reflect inhibition of target.

In cancer cells, the CDK-Rb-E2F axis is frequently deregulated, leading to uncontrolled cell division and progression. Alterations in cyclins and their CDKs, as well as inactivating mutations in *RB1*, could lead to increased E2F activity and higher S-phase fraction in tumor cells [4]. Various factors are responsible for upregulation of this axis, including *CCND* gene amplification [5], cyclin D overexpression [6], *CDK4/6* mutation/amplification [7], and loss of negative regulators of the complex, such as *CDKN2A* and *CDKN2B* [8]. Breast cancer is an example of a tumor that presents with deregulation of the cyclin pathway. Multiple studies suggest that, in hormone receptor–positive (HR+) breast cancer, cyclin pathway activation may lead to resistance to traditional endocrine therapy [9]. Indeed, inhibitors of CDK4/6 were clinically tested in patients with HR+ breast cancer and led to consistent benefit when administered with aromatase inhibitors [10–12]. Palbociclib, ribociclib, and abemaciclib are now U.S. Food and Drug Administration–approved CDK4/6 inhibitors for advanced breast cancer [13].

Despite the recent success of CDK inhibitors in breast cancer, biomarkers are lacking that help identify which patients are likely to derive benefit from these treatments. In addition, primary and acquired resistance to CDK inhibitors can be mediated by genomic alterations in genes involved in this pathway, such as *RB1* and *CCNE1* [14]. Both genes can be classified as potential resistance alterations related to CDK inhibition. Using biomarker knowledge for development and approval of targeted therapies is associated with higher therapeutic success [15, 16]. As previously demonstrated, many solid tumors harbor genetic alterations in cyclin pathway genes, including *CCN* amplifications and *CDKN2A* and *CDK4/6* aberrations [5, 17–19]. Cyclin inhibitors are in development for a variety of solid tumors with the strategy of selecting patients based on genomic characterization of the pathway [20, 21]. Hence, comprehensive characterization of the cyclin pathway alterations in the pancancer setting is needed.

Herein, we identified molecular alterations in genes involved in the cyclin activation/sensitizing pathway, as well as coexisting resistance and hormone pathway alterations, in 190,247 diverse solid tumors that underwent next-generation sequencing (NGS) in a Clinical Laboratory Improvement Amendment (CLIA)–certified laboratory.

Materials and Methods

Tissue Sampling

Consecutive samples submitted by thousands of physicians worldwide were analyzed using a CLIA-certified laboratory (Foundation Medicine, https://www.foundationmedicine.com). Tissue diagnoses were designated according to the pathology report and further verified by a pathologist at Foundation

Medicine. DNA was extracted from formalin-fixed, paraffin-embedded tissue, as previously described [22]. Patient identification was redacted for the study. Approval for the Foundation Medicine cohort, including a waiver of informed consent and Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (protocol no. 20152817).

Next-Generation Sequencing

DNA was extracted from formalin-fixed, paraffin-embedded sections, and comprehensive genomic profiling was performed on hybridization-captured, adaptor ligation-based libraries to a median depth of coverage of >500× [22]. The platform simultaneously sequenced the coding regions of 315 cancer-related genes plus introns from 28 genes often rearranged or altered in cancer. Alterations captured by NGS included base pair substitutions, insertions/deletions (both short and long), copy number alterations, and rearrangements.

Clustering of Genomic Alterations and Tumor Types

Genomic alterations of interest were classified either as activators of the cyclin pathway (eight genes, including *CDK4* amplification, *CDK6* amplification, *CCND1*, *CCND2*, *CCND3*, *CDKN2B* [loss], *CDKN2A* [loss], and *SMARCB1*) or as related to potential resistance pathways related to CDK4/6 inhibition (*RB1* and *CCNE1*). Additionally, genomic alterations in pathways related to cyclins (crosstalk pathways or targetable with drugs developed in combination with cyclin inhibitors, including *SMAD3, CDKN1A, CDKN1B, CDKN1C*, estrogen w? >receptor 1 [*ESR1*], and androgen receptor [*AR*]) were analyzed (supplemental online Table 1). Analysis of frequencies were performed by disease ontologies clustered according to American Joint Committee on Cancer 8th edition [23] and consistent with the tumor histologies on the submitted pathology report.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism, Python 2.7, and Anaconda version 4-4.3.21 (Anaconda, Austin, TX). Co-occurrence analysis was performing matching cyclin pathway genomic alterations with three different subsets of genomic alterations (resistance pathway, cyclin-related, and *ESR1*/*AR*).

Results

Alterations in any cyclin pathway activating/sensitizing genes (supplemental online Table 1) were found in 24% of the 190,247 tumors analyzed (Fig. 1). The most frequent alterations were *CDKN2A* loss (13.9%), *CDKN2B* loss (12.5%), and *CCND1* amplification (4.8%). *CDK4* and *CDK6* alterations were detected in 3% and 1.5% of samples, respectively. Overall, 89% of cases presented a single genomic alteration in one of the eight activating/sensitizing genes selected as part of the cyclin pathway (*CDK4* amplification, *CDK6* amplification, *CCND1, CCND2, CCND3, CDKN2B* [loss], *CDKN2A* [loss], *SMARCB1*). Alterations in two cyclin pathway activating/sensitizing genes occurred in 20% cases, and 1% of cases had more than two co-occurring alterations. The frequency of cyclin pathway activating/sensitizing alterations varied by histology and tumor type (Fig. 2A and C).

Characteristics of Cyclin Gene Alterations

Different types of alterations were identified in the eight cyclin pathway activating/sensitizing genes (*CDK4* amplification, *CDK6* amplification, *CCND1, CCND2, CCND3, CDKN2B* [loss], *CDKN2A* [loss], *SMARCB1*) (supplemental online Table 2). Copy number changes were the sole type of alteration detected in *CDK4, CDK6*, and *CCND1* genes (all amplifications). *CDKN2A* was uniformly affected by gene loss, whereas 1% of *CDKN2B* alterations were rearrangements. In fact, seven of the eight cyclin genes presented mostly (or exclusively) with copy number changes (*CDK4* amplification, *CDK6* amplification, *CCND1, CCND2, CCND3, CDKN2B* [loss], *CDKN2A* [loss]). A single nucleotide change was the predominant *SMARCB1* alteration (73% of cases of altered *SMARCB1*). *SMARCB1* was the most frequently rearranged gene of the pathway (7% of altered *SMARCB1*). Of the other genes included in this analysis, *RB1* (67% of cases altered *RB1*), *ESR1* (79%), *CDKN1A* (95%), and *CDKN1B* (82%) presented more frequently with single nucleotide changes. *CDKN2C* and *AR* presented more frequently with copy number changes (54% and 59% of cases with alterations, respectively).

Cyclin Activating/Sensitizing (*CDK4* Amplification, *CDK6* Amplification, *CCND1*, *CCND2*, *CCND3*, *CDKN2B* [Loss], *CDKN2A* [Loss], *SMARCB1*) Alterations by Histology

All 17 histologies demonstrated cyclin activating/sensitizing pathway alteration in all genes included as part of the pathway (except for the absence of *CCND3* alterations in gastrointestinal stromal tumors) (Fig. 2A, B and Fig. 3A).

Significant variability was seen in the patterns of cyclin activating/sensitizing alterations between different disease ontologies. Gliomas (54% of tumors had cyclin activating/sensitizing pathway alterations) and urothelial carcinoma (41%) were the histologies that most frequently harbored alterations; adenoid cystic (7%) and small cell carcinoma (6%) were the least commonly altered. Neuroendocrine carcinoma (which is in the same nosologic spectrum as small cell carcinoma) was also among the tumor histologies with a lower frequency of cyclin activating/sensitizing alterations (12%).

CDKN2A and *CDKN2B* deletions were the most frequent cyclin activating/sensitizing alterations across histologies, with similar frequencies between both genes in each histology (Fig. 3A). Although gliomas are associated with high frequencies of cyclin activating/sensitizing alterations, *CCND1* (0.3% of cases of gliomas), *CCND3* (0.2%), and *SMARCB1* (0.7%) were rarely altered in gliomas compared with other histologies. Alteration frequencies of note by histology include a high frequency of *CCND1* amplification in urothelial carcinoma (12.3%) and squamous cell carcinoma (13%), a high frequency of *CDK4* alterations in sarcomas (10.4%), and a relatively high proportion of *CCND2* alterations in germ cell tumors (16.3%, compared with 1.5% in the overall population).

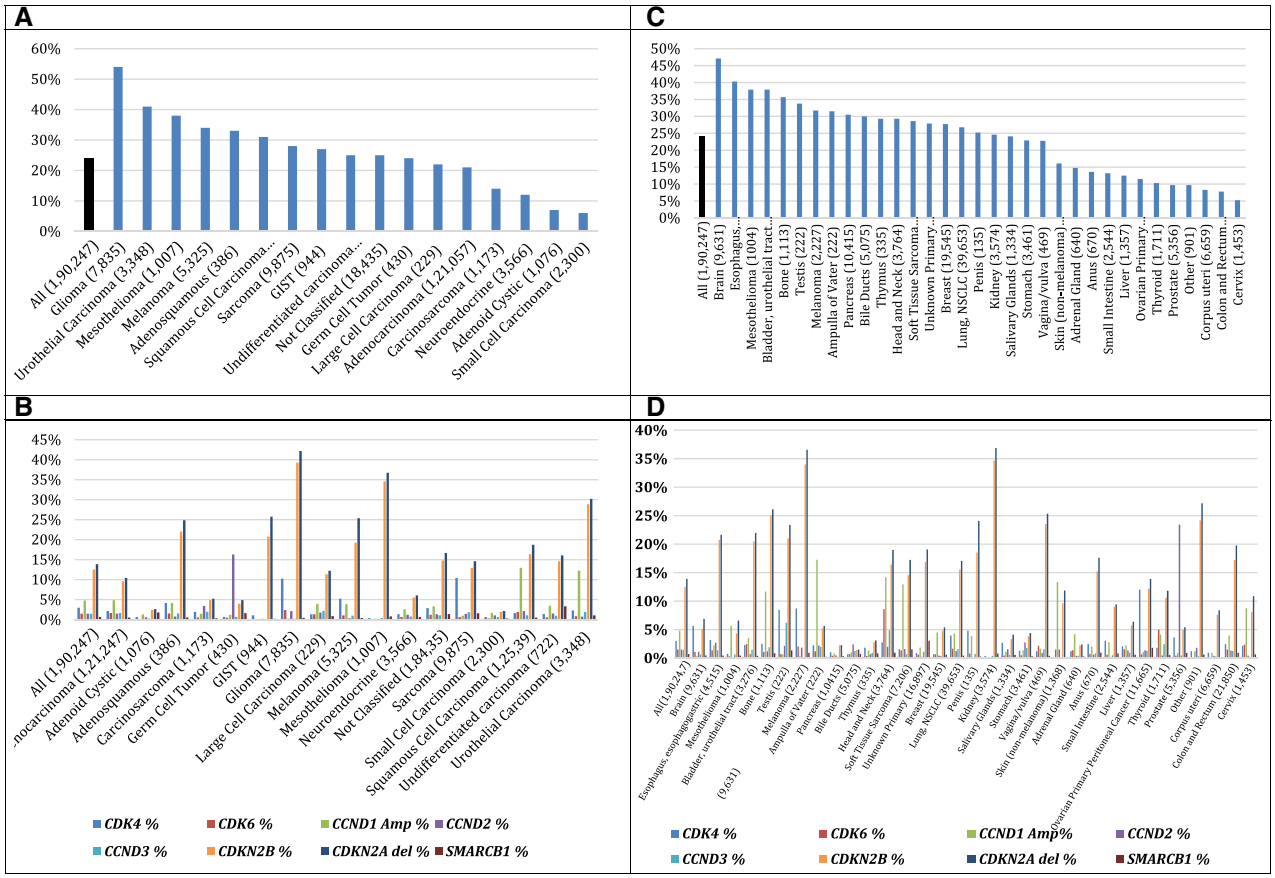

Figure 2. Cyclin pathway gene alterations in patients with cancer. Percent of patients with alterations is shown on the *y*-axis. Analysis of alteration frequency (%) is calculated as harboring at least one alteration per case. Numbers in brackets represent numbers of patients. **(A):** Analysis of overall alterations by histopathology. "All" represents all samples, regardless of histology (Fig. 3A). **(B):** Specific gene alteration frequencies by histopathology (Fig. 3A). **(C):** Analysis of overall alterations by disease type (Fig. 3B). **(D):** Specific gene alteration by disease type. Other includes parathyroid carcinoma, placenta choriocarcinoma, spine ependymoma, soft tissue paraganglioma, spine glioma, eye tumors, heart tumors, neuroblastoma, mediastinal neoplasias, pineal tumor, schwannoma, spleen sarcoma, scrotum tumors, and tracheal carcinomas (Fig. 3B).
Abbreviations: GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

Cyclin Activating/Sensitizing (*CDK4* Amplification, *CDK6* Amplification, *CCND1*, *CCND2*, *CCND3*, *CDKN2B* [Loss], *CDKN2A* [Loss], *SMARCB1*) Alterations by Disease Type

The top five of the 33 disease types analyzed harboring any type of cyclin activating/sensitizing alterations were brain (47.1% of cases of brain tumors had a cyclin alteration), esophageal (40.3%), mesothelioma (37.9%), bladder (37.9%), and primary bone cancers (35.7%) (Fig. 2C, D and Fig. 3B). Except for *CDK4* in thymic cancer and *CCND3* and *SMARCB1* in penile cancer, all other genes were altered in at least one case in each disease type. Cyclin gene alterations were less frequently detected in cervical (5.2%), colorectal (7.8%), uterine (8.3%), and prostate (9.7%) cancers.

Comparing the overall frequency of each gene with the specific disease types, some alterations were typical in some tumors (Fig. 2C, D and Fig. 3B). Breast cancer was the leading tumor for *CCND1* amplification (17.3% of cases vs. 4.8% overall for all tumors), soft tissue sarcomas for *CDK4* alterations (12% vs. 3% overall), esophageal cancer for *CDK6* alterations (8.6% vs. 1.5% overall), testicular cancers typically presented *CCND2* alterations (23.4% vs. 1.5% overall), bone tumors a high frequency of *CCND3* alterations (6.2% vs. 1.4% overall), and, finally, kidney cancers a relevant frequency of *SMARCB1* alterations (3% vs. 0.7% overall).

We discerned interesting information in uncommon tumors (supplemental online Table 3). Bladder (41%) and esophageal (45.5%) squamous cell carcinomas and malignant peripheral nerve sheath tumors (46.4%) harbored *CDKN2A* alterations. Breast neuroendocrine carcinoma presented a high frequency of *CCND1* amplification (26.3%). Overall, 38.1% of heart sarcomas had *CDK4* alterations, and 14.1% had *CCND3* alterations. Finally, although *SMARCB1* alterations are rare in the overall population (0.7%), some rare tumors presented high frequencies of alterations in this gene, including brain rhabdoid tumor (88.4%), kidney rhabdoid tumor (90%), kidney medullary carcinoma (41.3%), epithelioid sarcoma (56%), and extrarenal rhabdoid tumor (63.6%).

A

Histology	% Cases with activating/ sensitizing cyclin gene alterations	*CDKN2A* del	*CDKN2B* loss	*CCND1* amp	*CDK4*	*CDK6*	*CCND2*	*CCND3*	*SMARCB1*
All (190247)	**24**	**13.9**	**12.5**	**4.8**	**3.0**	**1.5**	**1.5**	**1.4**	**0.7**
Glioma (7835)	54	42.2	39.3	0.3	10.2	2.4	2.1	0.2	0.4
Urothelial Carcinoma (3348)	41	30.2	28.9	12.3	2.3	0.8	0.7	1.9	1.0
Mesothelioma (1007)	38	36.7	34.6	0.2	0.4	0.1	0.1	0.4	0.8
Melanoma (5325)	34	25.4	19.2	3.9	5.2	1.1	0.3	1.0	0.4
Adenosquamous (386)	33	24.9	22.0	4.1	4.1	1.6	0.8	1.6	0.5
Squamous Cell Carcinoma (12539)	31	18.7	16.3	13.0	1.6	1.9	2.1	1.1	0.5
Sarcoma (9875)	28	14.6	12.9	1.0	10.4	0.6	1.4	1.8	1.6
GIST (944)	27	25.7	20.8	0.1	1.1	0.0	0.2	0.0	0.2
Undifferentiated carcinoma (722)	25	16.1	14.5	3.5	1.4	0.6	1.5	1.0	3.3
Not Classified (18435)	25	16.7	14.8	3.3	2.9	1.2	1.3	1.1	1.4
Germ Cell Tumor (430)	24	4.9	4.0	1.2	0.7	0.5	**16.3**	0.7	1.6
Large Cell Carcinoma (229)	22	12.2	11.4	3.9	1.3	1.3	1.7	2.2	0.9
Adenocarcinoma (121057)	21	10.4	9.6	4.8	2.2	1.6	1.5	1.6	0.5
Carcinosarcoma (1173)	14	5.2	4.9	1.4	2.0	0.5	3.4	2.0	0.3
Neuroendocrine (3566)	12	6.0	5.5	2.6	1.3	0.7	1.2	0.8	0.6
Adenoid Cystic (1076)	7	2.6	2.4	1.3	0.7	0.1	0.6	0.3	1.8
Small Cell Carcinoma (2300)	6	2.1	2.0	1.7	0.7	0.3	1.0	0.6	0.3

B

Tumor Site	% Cases with activating/ sensitizing cyclin gene alterations	*CDKN2A* del	*CDKN2B* loss	*CCND1* amp	*CDK4*	*CDK6*	*CCND2*	*CCND3*	*SMARCB1*
All (190247)	**24.0**	**13.9**	**12.5**	**4.8**	**3.0**	**1.5**	**1.5**	**1.4**	**0.7**
Brain (9631)	47.1	**36.6**	34.0	0.3	8.7	2.0	1.8	0.2	0.9
Esophagus, esophagogastric junction (4515)	40.3	19.0	16.4	14.2	2.7	**8.6**	2.0	4.9	0.9
Mesothelioma (1004)	37.9	36.9	34.7	0.2	0.4	0.1	0.1	0.4	0.8
Bladder, urothelial tract (3276)	37.9	26.1	25.1	11.7	2.5	1.1	1.2	1.9	0.9
Bone (1113)	35.7	23.4	21.0	0.6	8.4	0.7	2.2	**6.2**	1.3
Testis (222)	33.8	5.4	5.0	0.9	3.6	0.5	**23.4**	0.5	0.9
Melanoma (2227)	31.7	24.1	18.5	3.9	4.8	0.8	0.2	0.7	0.3
Ampulla of Vater (222)	31.5	21.6	20.7	2.3	3.2	1.4	2.7	1.4	0.5
Pancreas (10415)	30.5	25.3	23.5	1.6	1.2	2.2	0.9	1.6	0.4
Bile Ducts (5075)	30.0	22	20	3.5	2.3	2.4	0.7	1.5	0.4
Thymus (335)	29.3	27.2	24.2	1.2	1.2	0.0	1.8	0.3	0.6
Head and Neck (3764)	29.3	17.2	14.5	12.9	1.5	1.4	1.6	0.7	1.5
Soft Tissue Sarcoma (7206)	28.6	13.9	12.2	1.1	**12.0**	0.7	1.4	1.3	1.8
Unknown Primary (16897)	27.9	19.8	17.2	4.0	2.4	1.5	1.3	1.3	0.9
Breast (19545)	27.7	5.6	5.2	**17.3**	2.2	1.2	2.2	2.0	0.2
Lung, NSCLC (39653)	26.8	17.0	15.5	4.3	4.0	1.6	1.2	1.6	0.4
Penis (135)	25.2	11.9	9.6	13.3	0.0	1.5	1.5	0.0	0.0
Kidney (3574)	24.6	19.1	16.9	1.8	0.9	0.6	0.2	1.1	**3.0**
Salivary Glands (1334)	24.1	17.6	15.2	2.0	2.5	0.7	0.6	0.8	1.0
Stomach (3461)	22.9	11.8	10.6	4.1	1.8	5.0	0.5	2.5	0.5
Vagina/vulva (469)	22.8	10.9	8.1	8.7	2.1	2.3	0.4	0.2	0.6
Skin (non-melanoma) (1368)	16.1	9.4	9.0	2.8	3.1	0.6	0.2	0.5	0.8
Adrenal Gland (640)	14.8	6.9	5.2	0.3	5.6	1.1	1.1	0.6	0.5
Anus (670)	13.6	6.6	4.3	5.7	0.7	0.3	0.1	0.6	0.4
Small Intestine (2544)	13.2	6.4	5.7	2.2	2.0	1.5	1.3	0.9	0.6
Liver (1357)	12.5	5.4	4.8	4.5	0.7	0.7	0.4	0.4	0.6
Ovary, Primary Peritoneal Cancer (11665)	11.5	4.4	3.8	1.4	1.3	0.5	2.8	1.8	0.3
Thyroid (1711)	10.3	8.4	7.6	0.9	0.9	0.1	0.4	0.3	0.1
Prostate (5356)	9.7	2.4	2.2	4.2	1.2	1.4	0.4	0.4	0.3
Other (901)	9.7	4.1	3.3	1.1	2.7	0.4	1.6	0.6	0.6
Corpus uteri (6659)	8.3	3.1	2.8	1.3	1.8	0.4	0.7	0.9	0.8
Colon and Rectum (21850)	7.8	1.5	1.4	1.0	0.6	0.7	2.4	1.3	0.7
Cervix (1453)	5.2	2.3	2.2	0.8	1.0	0.4	0.5	0.2	0.3

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Analysis of Potential Cyclin Pathway Resistance Genes (*RB1* and *CCNE1*)

We analyzed genomic alterations in *RB1* and *CCNE1* because they may promote resistance to cyclin inhibitors. Overall, *RB1* alterations were detected in 7.2% of samples; *CCNE1*, in 3.6% (supplemental online Figs. 1 and 2). In only 3% of cases were these alterations present simultaneously. Tumors presenting a high frequency of *RB1* alterations included bladder cancer (20.9%), nonmelanoma skin cancer (17.9%), soft tissue sarcomas (14.6%), and bone tumors (11.8%). In the case of *CCNE1*, alterations were frequent in ovarian (12.7%), esophageal (10.3%), and uterine cancers (9%). Although some disease types presented low frequencies of alterations of *RB1* and *CCNE1*, all had alterations.

We also analyzed the likelihood of co-occurrence of an alteration in the cyclin activation/sensitizing pathway and in a possible resistance pathway (*RB1* and *CCNE1*) by disease type (Fig. 4A and Table 1). In three diseases, we identified a higher likelihood of a co-occurrence of alterations in both pathways compared with an isolated alteration: colorectal cancers (odd ratio [OR], 1.53; $p < .001$), cervical cancer (OR, 1.29; $p < .001$), and small intestine (OR, 1.28; $p < .001$). In all other diseases analyzed, we detected lower likelihood of co-occurrence compared with an isolated alteration in cyclin activating/sensitizing and resistance pathway.

Co-Occurrence of Cyclin Activating/Sensitizing (*CDK4* Amplification, *CDK6* Amplification, *CCND1*, *CCND2*, *CCND3*, *CDKN2B* [Loss], *CDKN2A* [Loss], *SMARCB1*) Alterations and Related Pathway (*SMAD3*, *CDKN1A*, *CDKN1B*, *CDKN2C*) or Hormone Receptor Alterations

We analyzed the frequency of genes related to cyclin pathway (Fig. 1), including *SMAD3*, *CDKN1A*, *CDKN1B*, and *CDKN2C*. Overall, any of these genes were altered in 1% of cases (supplemental online Fig. 3). In comparison with other histologies, a higher frequency of *CDKN1A* alterations was found in urothelial carcinomas (3.5%), *CDKN1B* in neuroendocrine cancers (3.1%), and *CDKN2C* in gliomas (2.5%). *SMAD3* was rarely altered, regardless of the histology (supplemental online Fig. 4A). As for disease type, a notable finding was a high frequency of alterations in these genes in tumors from the ampulla of Vater compared with other sites (46% vs. 1%); relevant genes altered in this site were *CDKN1B* (20.7%) and *CDKN2C* (21.6%). For all tumors, there is a slightly higher likelihood of co-occurrence between alterations in cyclin-related genes (*SMAD3, CDKN1A, CDKN1B, CDKN2C*) and activating/sensitizing genes of the cyclin pathway (OR, 1.11; $p < .001$) (vs. an alteration in a cyclin-related gene in the presence of wild-type activating/sensitizing cyclin genes) (Fig. 4B and Table 2); however, substantial variation appeared when individual tumor types were analyzed. Tumors with a higher likelihood of co-occurrence included primary bone (OR, 3.61; $p < .001$) and brain cancers (OR, 3.00; $p < .001$), whereas a higher likelihood of an isolated alteration was detected in breast (OR, 0.48; $p < .001$) and prostate tumors (OR, 0.51; $p < .001$).

We also analyzed the frequency of hormone pathway–related genes, including *ESR1* and *AR* (supplemental online Figs. 3 and 5). Overall, *ESR1* was altered in 1.5% of tumors. Higher frequencies of alterations were noted in breast (11% of breast cancers had an *ESR1* alteration) and uterine cancers (3.6%). *AR* was altered in 0.9% of tumors, and, as expected, prostate cancer presented a high frequency of alterations (20.9%). Surprisingly, *AR* was also altered (5.7%) in undifferentiated carcinomas. Co-occurrence of cyclin activating/sensitizing genes and hormone altered genes was analyzed. Diseases with at least 1% of prevalence of hormone receptor alterations are reported (Fig. 4C and supplemental online Table 4). Cervical cancers presented an incidence of 0.6% of *ESR1* alterations and 0.3% of *AR* alterations. These alterations presented significant positive co-occurrence likelihood with cyclin activating/sensitizing alterations (OR, 4.08; $p = .04$). In breast cancer we also detected a higher likelihood of co-occurrence of *ESR1* and cyclin activating/sensitizing alterations (OR, 1.63; $p < .001$) (vs. *ESR1* alteration in the presence of wild-type cyclin activating/sensitizing genes), whereas in prostate cancer a significant co-occurrence between *AR* and cyclin activating/sensitizing alterations was detected (OR, 1.79; $p < .001$) (vs. *AR* alteration in the presence of wild-type cyclin activating/sensitizing genes).

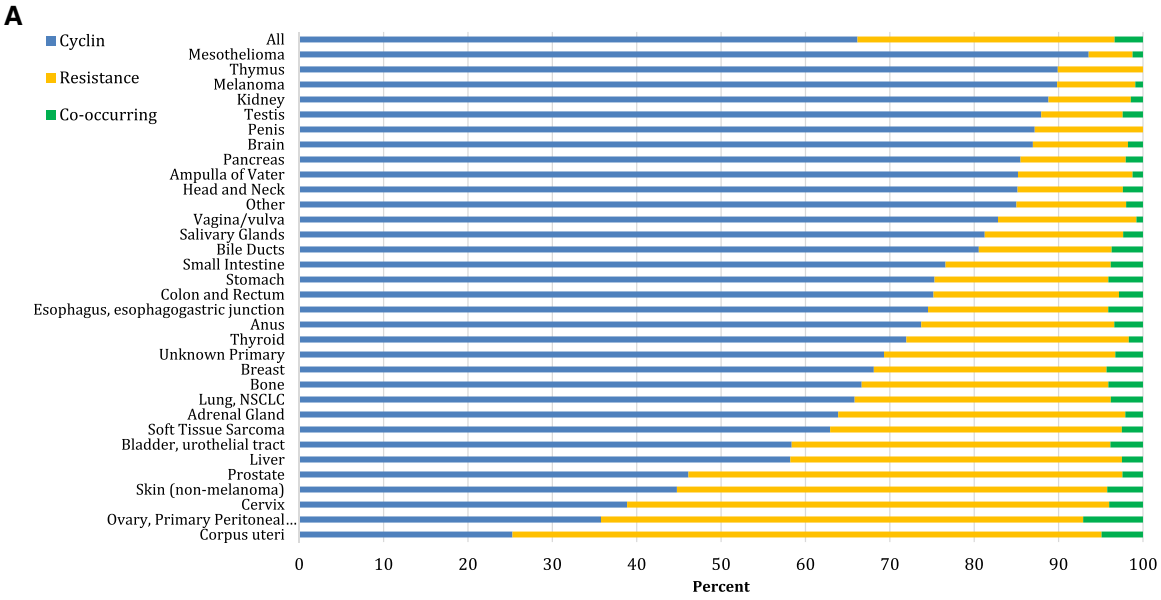
Discussion

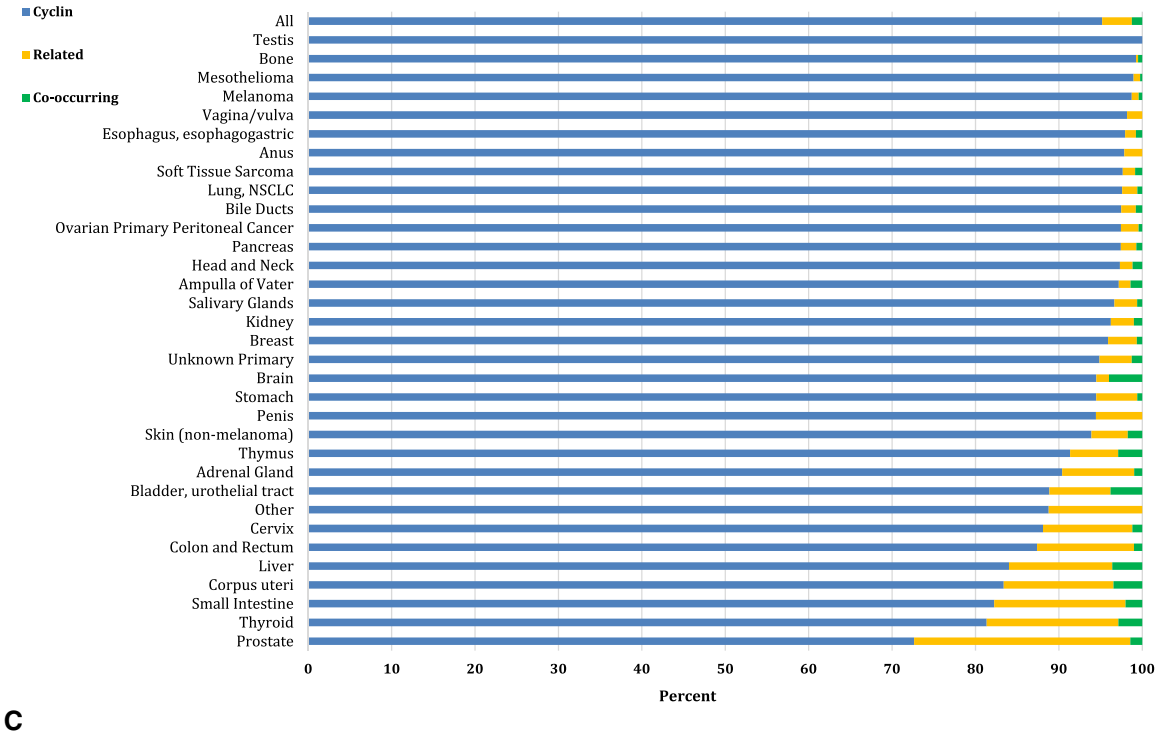
The cyclin pathway is frequently altered in cancer and may present targeted therapy opportunities. This study represents the largest series ($n = 190{,}247$) describing the landscape of genomic abnormalities in different cyclin genes. Overall, we demonstrated that 24% of tumors harbor alterations in genes related to activation/sensitization of the pathway, whereas 10% presented alterations that could lead to resistance to cyclin inhibition (Fig. 1). The frequency of alterations of cyclin genes varied by disease, being highest in brain tumors (47%), esophagogastric cancers (40%), and mesotheliomas (38%), and by histopathology, with highest frequencies in gliomas (54%) and urothelial cancers (41%).

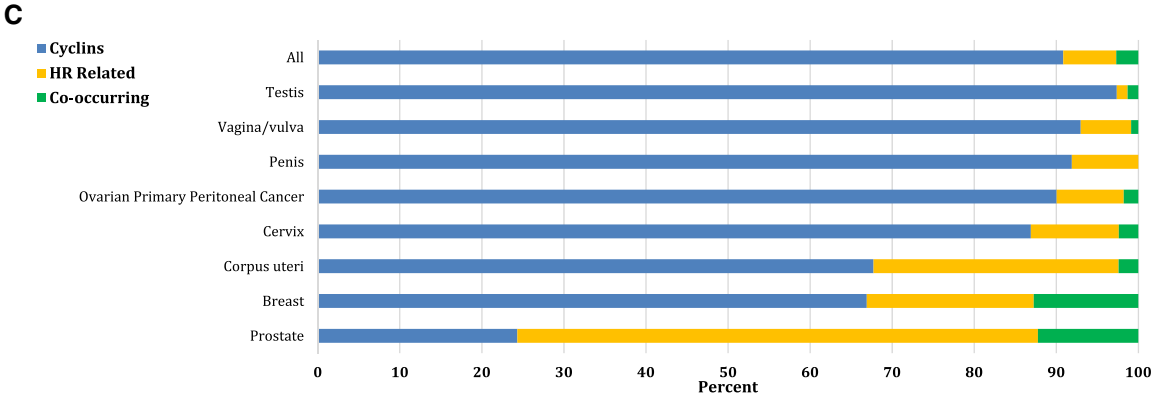
A previous report from our group using a similar genomic analysis, albeit with only 4,864 patients, similarly revealed frequent cyclin gene alterations across cancers [18], as did data on cBioPortal (http://www.cbioportal.org) and other prior smaller series [5, 17].

Unique to this study, *SMARCB1* alterations were analyzed as part of the cyclin pathway. In fact, *SMARCB1* represses cyclin D1 and inhibits the action of CDK4 resulting in hypophosphorylation of Rb [24, 25]. Overall, we detected alterations in this gene in 0.7% of patients, whereas Memorial Sloan Kettering-Integrated mutation profiling of actionable

Figure 3. Chart of alterations (%) in cyclin pathway genes. The percentages of patients with an alteration are shown. **(A):** Alterations are categorized by histopathologic subtype (this chart corresponds to Fig. 2A and B). **(B):** Alterations are categorized by disease type (this chart corresponds to Fig. 2C and D). On both panels, pink denotes percentage of patients with alteration above median and yellow denotes percentage below median; those without color are 0%; colored yellow 0% are between 0.001% and 0.5%.
Abbreviations: amp, amplification; del, deletion; GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer.







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cancer targets (MSK-IMPACT) demonstrated an alteration frequency of 1.3% (*n* = 10,945) [26]. Mutations in *SMARCB1* were first described in malignant rhabdoid tumors [27]. We demonstrated a high frequency of *SMARCB1* alterations in tumors with a rhabdoid component (supplemental online Table 3), including brain teratoid rhabdoid tumor (88.4% with alterations), kidney malignant rhabdoid tumor (90%), and extrarenal rhabdoid tumors (63.6%). *SMARCB1* inactivation was previously also demonstrated as a characteristic hallmark of renal medullary carcinomas in four cases [28]. Using an NGS approach, we found that 41.3% of these tumors had *SMARCB1* genomic alterations (*n* = 46). Although quite uncommon, *SMARCB1* alterations can be detected in a variety of other tumors based on our analysis, especially those neoplasms with a mesenchymal component. Small subsets of tumors can be driven by complete loss of *SMARCB1* [29], which offers targeted therapy opportunities, including with CDK4, enhancer of zeste homolog 2, and proteasome inhibition [24, 30].

The large numbers of samples in our series allowed for other interesting observations at both gene and disease levels. *CCND2* may be deregulated in testicular germ cell tumors [31], and we demonstrated a high frequency of alterations in *CCND2* (mainly amplifications) in germ cell tumors of different origins (23.4% vs. 1.5% overall). About 8.6% of esophageal cancers harbor alterations in *CDK6* in our series, which may identify a possible subset of these patients who are resistant to radiotherapy and may be candidates for therapeutic effects of CDK4/6 inhibition [32, 33]. Sarcomas are a heterogeneous group of different tumor subtypes and by and large have been devoid of advances in treatment of systemic disease. Identification of small genomic-driven subsets is a valid strategy for the treatment of these patients [34]. Regarding the cyclin pathway, soft tissue sarcomas were enriched for *CDK4* alterations (12% vs. 3% overall), especially heart sarcomas (38.1% tumors presented alterations); conversely, bone tumors had a high frequency of *CCND3* alterations (6.2% vs. 1.4% overall). Cyclin inhibition can be further explored in sarcomas, but patient selection will be essential for therapeutic success [35–37].

The current regulatory approvals for all three CDK4/6 inhibitors are for hormone-positive breast cancer, regardless of genomic biomarkers. Exploratory analysis of prospective trials in breast cancer demonstrated that the efficacy of palbociclib was not modulated by *CCND1* amplification and cyclin D1, CDK4, or CDK6 expression [38–40]. For other solid tumors, a phase II basket trial with ribociclib included heavily pretreated patients with advanced cancer and a cyclin genomic alteration (either *CDK4/6* mutation or amplification, *CCND1/3* amplification, or *CDKN2A* mutation or loss). Of 106 patients, only three experienced partial responses (soft tissue sarcoma, urothelial carcinoma, and adenocarcinoma) [20]. A prospective trial with palbociclib in patients with pancreatic or biliary cancers with *CDKN2A* loss or mutation (prevalence in our series of 25.3% and 22%, respectively) also failed to demonstrate activity [21]. So far, these data suggest that targeting cyclin pathway with CD4/6 inhibitors in monotherapy is challenging. Further understanding of genomic co-alterations in tumors is needed, and as a requisite to that, broad-based genomic profiling of known sensitivity and resistance determinants as well as exploratory analyses are required [5]. In prostate cancer, crosstalk between androgen signaling and cyclin pathway was suggested, as well as AR independency mediated by cyclin activation [41, 42]. Interestingly, we report a co-occurrence of *AR* and cyclin gene alterations, which could identify a subset of patients with more intense resistance to next-generation antiandrogens. Preclinical rationale suggests further testing of CDK4/6 inhibitors in this setting, as activity of these agents is independent of *AR* alterations [43]. It is also noteworthy that, for the first time, we report a significant co-occurrence of *ESR1* mutations with cyclin pathway alterations for breast cancer (OR, 1.63; $p < .001$). Strategies for these patients possibly include selective estrogen downregulators over aromatase inhibitors when combined with CDK4/6 inhibitors [44].

Resistance mechanisms to cyclin inhibition are also important in this setting. *Rb1* inactivating mutations may confer resistance to cyclin inhibition and may also emerge during therapy with palbociclib [45]. High *CCNE1* mRNA expression was also associated with resistance to this drug [40]. In our series, we demonstrated that possible genomic mechanisms of resistance can be detected in various tumors, especially bladder cancer (20.9%) and nonmelanoma skin cancer (17.9%) for *Rb1*, and ovarian (12.7%) and esophageal (10.3%) cancers for *CCNE1*. Interestingly, in the majority of tumors we detected a lower likelihood of co-occurrence of resistance and sensitizing mutations, which may be interesting for selection of patients for cyclin inhibitors. This finding was also suggested previously analyzing *Rb1* alterations [18].

It is important to note that several other genes may interact with the cyclin pathway and, thus, affect cell cycle progression. *TP53* is the most frequently mutated cancer gene (64% in our prior report [46]) and is responsible for regulating p21 (*CDKN1A*) levels via posttranslation mechanisms [47]. *MDM2/MDM4* activation can lead to p21 degradation, whereas *MDM2* amplification can not only

Figure 4. Co-alteration analysis. **(A):** Resistance pathway (*RB1* and *CCNE1*) and cyclin pathway co-incidence. The ratio of alterations in the cyclin pathway only, the resistance pathway only, or alterations in both the cyclin and resistance pathways is shown for all disease types with a significant association between the two pathways (*p* value ≤.05 for co-incidence; 33 disease types total had a significant association—Table 1). **(B):** Related genes (*SMAD3*, *CDKN1A*, *CDKN1B*, *CDKN2C*) and cyclin pathway co-incidence. The ratio of alterations in the cyclin pathway only, the related pathway only, or alterations in both the cyclin and related pathways is shown for all disease types with a significant association between the two pathways (*p* value ≤.05 for co-incidence; 33 disease types total had a significant association—Table 2). **(C):** Hormone receptor genes (estrogen receptor 1 [*ESR1*], androgen receptor [*AR*]) and cyclin pathway co-incidence. The ratio of alterations in the cyclin pathway only, the hormone receptor only, or alterations in both the cyclin and hormone pathways is shown for disease types in which prevalence of *AR* or *ESR1* alterations were at least 1%—supplemental online Table 3).
Abbreviations: HR, hormone receptor; NSCLC, non-small cell lung cancer.

Table 1. Co-occurrence of alterations in cyclin activating/sensitizing (*CDK4* amplification, *CDK6* amplification, *CCND1*, *CCND2*, *CCND3*, *CDKN2B* [loss], *CDKN2A* [loss], and *SMARCB1*) and resistance genes (*RB1* and *CCNE1*) in the cyclin pathway

Tumor type	Sensitizing alterations only	Resistant alterations only	Both sensitizing and resistant	Neither alteration	OR sensitizing alteration in resistant patients[a]	OR resistant alteration in sensitizing patients[b]	*p* value[c]
All	43,269	19,934	2,195	124,849	0.39	0.35	.0001
Adrenal gland	92	49	3	496	0.37	0.35	.0001
Ampulla of Vater	69	11	1	141	0.25	0.20	.0001
Anus	87	27	4	552	0.95	0.94	.0001
Bile ducts	1,453	284	67	3,271	0.62	0.55	.0001
Bladder, urothelial tract	1,164	753	77	1,282	0.19	0.17	.0001
Bone	374	164	23	552	0.30	0.25	.0001
Brain	4,442	575	91	4,523	0.28	0.18	.0001
Breast	5,100	2,065	323	12,057	0.46	0.41	.0001
Cervix	68	100	7	1,278	1.29	1.29	.0001
Colon and rectum	1,641	480	62	19,667	1.49	1.53	.0001
Corpus uteri	464	1,282	90	4,823	0.75	0.77	.0001
Esophagus, esophagogastric	1,723	494	95	2,203	0.37	0.29	.0001
Head and neck	1,071	157	30	2,506	0.54	0.46	.0001
Kidney	864	95	14	2,601	0.52	0.45	.0001
Liver	163	110	7	1,077	0.46	0.44	.0001
Lung, NSCLC	10,064	4,642	582	24,365	0.38	0.34	.0001
Melanoma	698	72	7	1,450	0.27	0.21	.0001
Mesothelioma	378	21	5	600	0.50	0.39	.0001
Other	85	13	2	801	1.39	1.44	.0001
Ovary/ peritoneal Cancer	1,117	1,785	221	8,542	0.95	0.96	.0001
Pancreas	3,104	453	74	6,784	0.45	0.37	.0001
Penis	34	5	0	96	0.00	0.00	.0463
Prostate	494	551	26	4,285	0.44	0.44	.0001
Salivary glands	312	63	9	950	0.51	0.45	.0001
Skin (nonmelanoma)	201	229	19	919	0.43	0.43	.0001
Small intestine	321	82	16	2,125	1.24	1.28	.0001
Soft tissue sarcoma	1,983	1,089	79	4,055	0.21	0.18	.0001
Stomach	753	206	41	2,461	0.71	0.67	.0001
Testis	73	8	2	139	0.58	0.49	.0001
Thymus	98	11	0	226	0.00	0.00	.0089
Thyroid	172	63	4	1,472	0.57	0.55	.0001
Unknown primary	4,500	1,779	213	10,405	0.35	0.31	.0001
Vagina/vulva	106	21	1	341	0.19	0.16	.0001

Only tumors with a statistically significant association are shown in this table.
[a]Odds ratio of a sensitizing cyclin gene alteration in patients with a resistant cyclin alteration compared with patients with only wild type for resistant cyclin genes.
[b]Odds ratio of a resistance cyclin alteration in patients with a sensitizing cyclin alteration compared with patients with only wild type for sensitizing cyclin genes.
[c]*p* value for co-occurrence test.
Abbreviations: NSCLC, non-small cell lung cancer; OR, odds ratio.

Table 2. Co-occurrence of alterations in the cyclin activating/sensitizing genes (*CDK4* amplification, *CDK6* amplification, *CCND1*, *CCND2*, *CCND3*, *CDKN2B* [loss], *CDKN2A* [loss], and *SMARCB1*) and related genes (*SMAD3, CDKN1A, CDKN1B, CDKN2C*)

Tumor type	Sensitizing alterations only	Related alterations only	Both sensitizing and related	Neither alteration	OR sensitizing alteration in related patients[a]	OR related alteration in sensitizing patients[b]	*p* value[c]
All	44,876	1,682	587	143,102	1.08	1.11	.0001
Ampulla of Vater	69	1	1	151	1.59	2.17	.0001
Bile ducts	1,508	27	12	3,528	1.03	1.04	.0001
Bladder, urothelial tract	1,190	98	51	1,937	0.90	0.85	.0001
Bone	395	1	2	715	1.87	3.61	.0001
Brain	4,349	69	184	5,029	1.57	3.00	.0001
Breast	5,387	194	36	13,928	0.56	0.48	.0001
Cervix	74	9	1	1,369	1.95	2.04	.0490
Colon and rectum	1,684	224	19	19,923	1.00	1.00	.0001
Corpus uteri	532	84	22	6,021	2.56	2.89	.0001
Esophagus, esophagogastric junction	1,804	24	14	2,673	0.91	0.87	.0001
Head and neck	1,088	17	13	2,646	1.49	1.85	.0001
Kidney	869	25	9	2,671	1.08	1.11	.0001
Lung, NSCLC	10,582	197	64	28,810	0.91	0.89	.0001
Melanoma	702	6	3	1,516	1.05	1.08	.0001
Mesothelioma	382	3	1	618	0.65	0.54	.0001
Ovary/ peritoneal cancer	1,332	29	6	10,298	1.50	1.60	.0001
Pancreas	3,155	60	23	7,177	0.91	0.87	.0001
Penis	34	2	0	99	0.00	0.00	.0103
Prostate	510	182	10	4,654	0.53	0.51	.0001
Salivary glands	319	9	2	1,004	0.75	0.70	.0001
Skin (nonmelanoma)	216	10	4	1,138	1.79	2.09	.0001
Soft tissue sarcoma	2,044	31	18	5,113	1.29	1.45	.0001
Stomach	789	41	5	2,626	0.47	0.41	.0001
Testis	75	0	0	147	Not calculated	Not calculated	.0001
Thymus	95	6	3	231	1.14	1.21	.0001
Thyroid	170	33	6	1,502	1.51	1.59	.0122
Unknown primary	4,651	189	62	11,995	0.88	0.85	.0001
Vagina/vulva	107	2	0	360	0.00	0.00	.0001

Only tumors with a statistically significant association are shown in this table.
[a]Odds ratio of a sensitizing cyclin gene alteration (in patients with a related cyclin gene alteration compared with patients with only wild type for cyclin-related genes).
[b]Odds ratio of a related gene alteration in patients with a sensitizing cyclin alteration compared with patients with only wild type for sensitizing cyclin genes.
[c]*p* value for co-occurrence test.
Abbreviations: NSCLC, non-small cell lung cancer; OR, odds ratio.

inactivate p53 but also cause Rb1 degradation and E2F1 transactivation [48]. All these events affect the ability of cells to progress from G1 into S phase and could modulate the activity of cyclin inhibitors.

This study has several limitations, including the lack of clinical correlates, which limits elucidating possible associations between genomic alterations and prognosis or response to therapies. The cyclin pathway regulation can also be affected

by epigenetic modulation or noncoding genomic alterations [49]. The FoundationOne CDx assay used for this report does not access these alterations, and, therefore, studies are needed to characterize the landscape of epigenetic alterations in the cyclin pathway.

Conclusion

Our analysis shows that alterations in cyclin pathway activating/sensitizing genes occurred in 24% of 190,247 tumors. Specific patterns of alterations differed between tumor types and between patients within any given cancer classification, suggesting the need for individualized characterization of cancers by NGS if these gene alterations are to be optimally exploited in the clinical therapeutic setting. These observations highlight the need for broad-based profiling of tumors from patients with advanced cancers.

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Disclosures

Denis L. Jardim: Roche, Janssen, Astellas, Merck Sharp & Dohme, Bristol-Myers Squibb, Libbs (H), Janssen, Bristol-Myers Squibb, Libbs (C/A); **Sherri Z. Millis:** Foundation Medicine (E, OI); **Jeffrey S. Ross:** Foundation Medicine (E, OI); **Michelle Sue-Ann Woo:** Daiichi Sankyo, Foundation Medicine (E, OI); **Siraj M. Ali:** Foundation Medicine (E, OI); **Razelle Kurzrock:** IDbyDNA, CureMatch, Inc., Soluventis (OI), Gaido, LOXO, XBiotech, Actuate Therapeutics, Roche, NeoMed, Soluventis, Pfizer, Merck (C/A), Roche (H), Incyte, Genetech, Merck Serono, Pfizer, Sequenom, Foundation Medicine, Guardant Health, Grifols, Konica Minolta, DeBiopharm, Boehringer Ingelheim, Omniseq (RF—institutional), CureMatch, Inc., CureMetrix, Inc. (other—board member).

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