Pan-Cancer Analysis of *CDK12* Loss-of-Function Alterations and Their Association with the Focal Tandem-Duplicator Phenotype

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. CDK12 • Tandem duplication • Immunotherapy • Genomics

Abstract

Background. *CDK12* loss-of-function (LOF) genomic alterations are associated with focal tandem duplications (FTDs) in ovarian and prostate cancers. Because these FTDs may produce fusion-induced neoantigens (FINAs), *CDK12* alteration is a candidate biomarker for immune checkpoint inhibitor sensitivity. Here we determine the prevalence of *CDK12*-LOF alterations and their association with FTDs across diverse tumor types.

Materials and Methods. A total of 142,133 tumor samples comprising 379 cancer types were sequenced (August 2014 to April 2018) by hybrid capture-based comprehensive genomic profiling (Foundation Medicine, Cambridge, MA) as part of routine clinical care. Results were analyzed for base substitutions, short insertions/deletions, rearrangements, and copy number alterations. *CDK12*-LOF genomic alterations were assessed for zygosity status and association with FTDs/focal copy number gain.

Results. *CDK12* genomic alterations were detected in 1.1% of all cases, most frequently in prostate cancer (5.6%), but were also observed at >1% frequency in 11 cancer types. Across multiple cancer types, including prostate, gastric/esophageal, ovarian, breast, and endometrial cancer, the number of FTDs was significantly increased in *CDK12*-LOF versus *CDK12* wild-type cases. Notably, *CDK12*-LOF was not consistently associated with a homologous recombination deficiency genomic signature. Quantitative assessment of *CDK12*-associated FTDs by measurement of single copy number gains identified novel likely deleterious *CDK12* kinase-domain mutations in prostate and ovarian cancers.

Conclusion. Detection of *CDK12*-LOF genomic alterations and their association with FTDs in a diverse spectrum of malignancies suggests that immunotherapy approaches targeting FINAs derived from *CDK12*-associated FTDs may be a broadly applicable strategy that could be explored across cancer types in a tumor-agnostic manner. *The Oncologist* 2019;24:1526–1533

Implications for Practice: *CDK12* inactivation in ovarian and prostate cancer results in the generation of focal tandem duplications, which can cause fusion-induced neoantigens. In prostate cancer, *CDK12* alterations have demonstrated promise as a potential predictive biomarker for response to immune checkpoint blockade. This study evaluated genomic profiling data from >142,000 tumors to determine the prevalence of *CDK12* loss-of-function genomic alterations across tumor types and demonstrated that *CDK12* alterations are associated with the tandem-duplicator phenotype in cancer types other than ovarian and prostate cancer. The association of *CDK12* alterations with focal tandem duplications across broad cancer types suggests that *CDK12* inactivation warrants further investigation as a pan-cancer biomarker for immunotherapy benefit.

Introduction

Recurrent loss-of-function (LOF) alterations in the *CDK12* tumor suppressor gene, which encodes cyclin-dependent kinase 12, have been described in prostate and ovarian cancers [1–4]. The CDK12-cyclin K heterodimer regulates RNA

polymerase II [5] and is implicated in transcriptional control of homologous recombination repair genes [6]. Therefore, *CDK12* genomic alteration (GA) has emerged as a potential attractive candidate biomarker for synthetic lethal targeting by poly (ADP-ribose) polymerase (PARP) inhibitors [7]. However, preliminary clinical evidence suggests that PARP inhibitor therapy may be largely ineffective in treatment of patients with advanced prostate cancer harboring *CDK12* GAs [8].

In ovarian and prostate cancers, *CDK12* loss-of-function (*CDK12*-LOF) alterations are not associated with a homologous recombination deficiency (HRD) phenotype but are instead associated with the tandem-duplicator phenotype (TDP), a genomic signature characterized by focal tandem duplications (FTDs) with a bimodal size distribution with modes of either 0.2–0.4 Mb or 1.7–3.0 Mb [1, 3, 9, 10]. *CDK12*-associated FTDs can result in expressed gene fusions and fusion-induced neoantigens (FINAs), raising the possibility of *CDK12*-LOF alteration as a predictive biomarker for immune checkpoint inhibitor (ICI) sensitivity [1]. In prostate cancer, *CDK12*-LOF alterations are associated with increased immune infiltrates, and some patients with *CDK12*-altered metastatic castration-resistant prostate cancer have derived clinical benefit from ICI therapy [1].

In this study, we assess comprehensive genomic profiling results from 142,133 cancer specimens to determine the prevalence of *CDK12*-LOF alterations and their association with FTDs in multiple tumor types beyond ovarian and prostate cancers. We hypothesized that *CDK12* inactivation would be observed at a reasonable frequency in many cancer types, potentially leading to a tumor-agnostic genomic biomarker of immunotherapy benefit.

MATERIALS AND METHODS

Approval for this study, 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 20152817). Tumor tissue specimens (*n* = 142,133; 44% metastatic site specimens) underwent comprehensive genomic profiling (CGP) using a validated hybrid capture-based next-generation sequencing (NGS) assay (FoundationOne) [11] in a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited, New York State-approved laboratory (Foundation Medicine, Cambridge, MA). CGP was performed on hybridization-captured, adaptor ligation-based libraries to a median coverage depth of 764× for exons of 395 cancer-related genes plus select introns from 31 genes frequently rearranged in cancer (supplemental online Table 1). Results were analyzed for base substitutions, short insertions/deletions (indels), rearrangements, and copy number alterations (amplification and homozygous deletion). Custom filtering was applied to remove benign germline variants as described [12]. *CDK12* GAs included protein-truncating mutations, homozygous deletions, genomic rearrangements, and likely pathogenic kinase-domain missense mutations (supplemental online Table 2); all other alterations were classified as variants of unknown significance (VUS). Zygosity status for mutations was determined as previously described [13]. Bi-allelic alterations were defined as (a) mutations with loss of heterozygosity (LOH) at the wild-type allele, as determined by zygosity status [13], (b) copy number loss (homozygous deletion), or (c) ≥2 *CDK12* GAs in a given sample. Percent genome-wide loss of heterozygosity (gLOH) was used as a surrogate marker of HRD status and was calculated as previously described, with a gLOH score of ≥16% being designated as gLOH-High [14]. FTD burden was assessed by counting the number of detected duplicating rearrangements per case. FTD-derived gene fusions were defined as FTDs that resulted in an in-strand juxtaposition of two coding sequences. For prostate cancer, the *TMPRSS2-ERG* duplication was excluded from the FTD burden score because this driver event has been shown to be independent of *CDK12* inactivation [1]. Single copy number gain (CN + 1) score was derived by evaluating the number of segments (between 10 kb and 10 Mb) with single copy gains relative to the median ploidy of the sample. CN + 1 score was assessable for cases that met copy number metrics based on the intersegment signal-to-noise ratios of normalized and GC corrected log-2-ratio data; samples with low signal-to-noise ratio were excluded from analysis.

RESULTS

To assess the prevalence of at least mono-allelic *CDK12* GAs across tumor types, we assessed CGP results from 142,133 tumors that were sequenced in the context of routine clinical care (Fig. 1A; supplemental online Fig. 1; supplemental online Table 3). Overall, *CDK12* GAs were observed in 1.1% of cases. As expected, *CDK12* GAs were most prevalent in prostate cancer (5.6%). Across other cancer types, *CDK12* GAs were observed at a 0.9% frequency overall, most commonly in vulvar/vaginal squamous cell carcinoma (4.0%), gastric/esophageal cancer (1.9%), ovarian cancer (1.9%), cervical cancer (1.7%), nonmelanoma skin cancer (1.7%), salivary gland cancer (1.5%), breast cancer (1.4%), endometrial cancer (1.3%), bladder/urothelial cancer (1.1%), and colorectal cancer (1.1%). In most other diseases, *CDK12* GAs were detected below a 1% prevalence.

To estimate the fraction of *CDK12* GAs likely to result in bi-allelic inactivation, we examined the relative fraction of *CDK12*-altered cases harboring each different class of GA (Fig. 1B); to ensure sufficient power to evaluate correlation of *CDK12* genotype with phenotype, cancer types with a raw count of ≥50 cases with *CDK12* GAs were chosen for in-depth analysis. As demonstrated, bi-allelic *CDK12* inactivation by copy number loss was most often observed in prostate (0.3% overall, 5.0% of *CDK12*-altered specimens) and ovarian cancers (0.05% overall, 2.4% of *CDK12*-altered specimens) but was not generally observed in other disease types (Fig. 1A, 1B). Short variant mutations were assessed for zygosity status to determine whether *CDK12* GAs were bi-allelic (pathogenic mutation, with LOH of the wild-type allele) or mono-allelic (heterozygous mutation); the ratio of bi-allelic to mono-allelic short variant mutations was highest in ovarian and breast cancers (Fig. 1B). Multiple *CDK12* GAs in a given tumor were presumed bi-allelic based on the association of such alterations with FTDs in prostate cancer [3]: 32.4% of prostate cancer cases with *CDK12* GAs harbored multiple alterations, but multiple alterations were also observed in
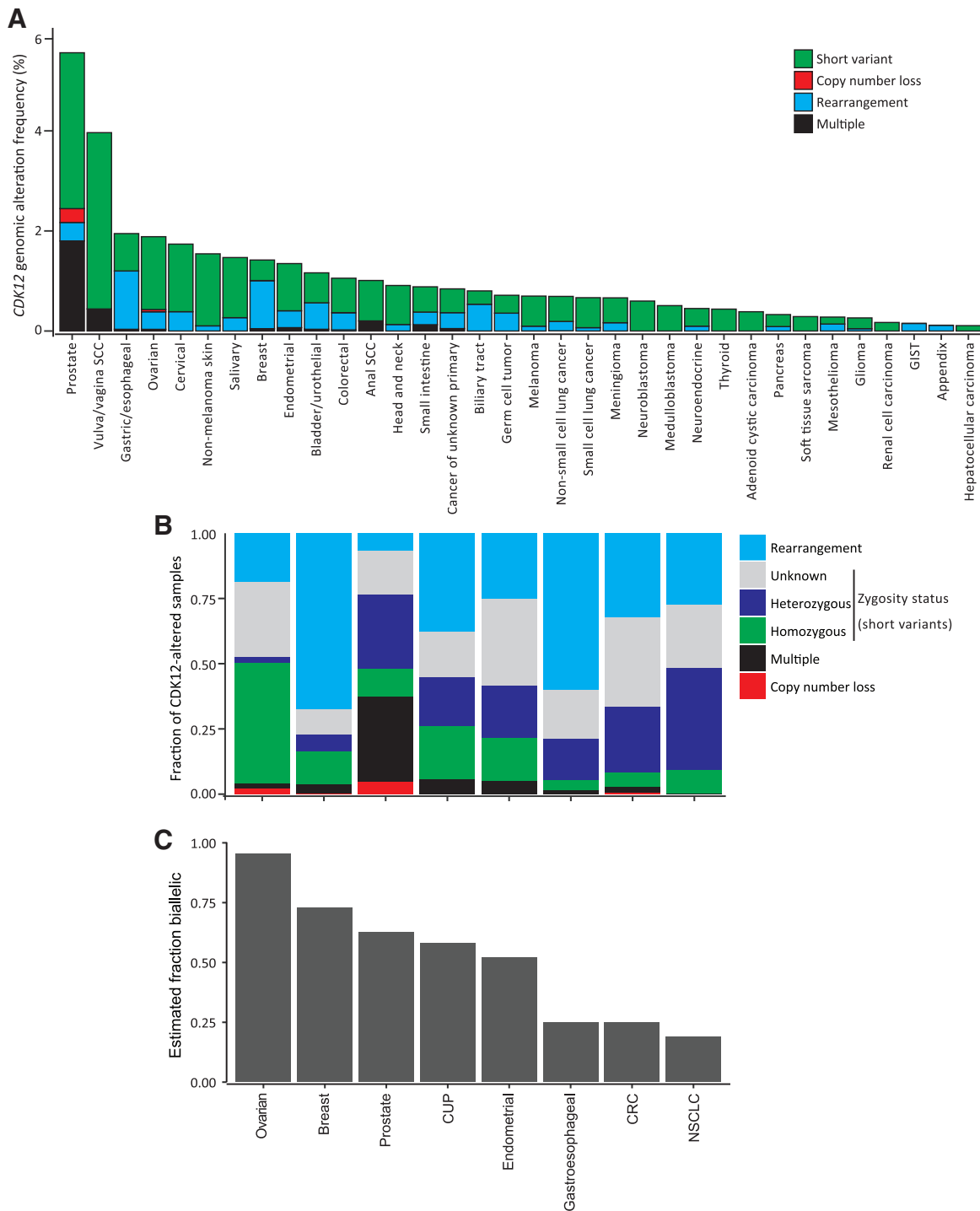
Figure 1. Pan-cancer analysis of *CDK12* alterations. **(A):** Frequency of *CDK12* loss-of-function (LOF) alterations across multiple tumor types. Cancer types included are those with at least 100 sequenced samples and with ≥1 case with *CDK12*-LOF alteration. Full details of cancer-type prevalence are included in supplemental online Table 3. "Multiple" indicates samples with ≥2 concurrent *CDK12* genomic alterations (GAs). **(B):** Relative fraction of cases with each class of *CDK12* alteration. Cancer types with ≥50 *CDK12* GA cases were individually assessed in this analysis. Short variant alterations were evaluated for zygosity. **(C):** Bi-allelic fraction was determined for cases where mono-allelic versus bi-allelic status could be determined. Bi-allelic alterations included copy number loss, mutation with wild-type copy under loss of heterozygosity, or multiple detected alterations in a given case. Mono-allelic mutations were those determined as heterozygous.
Abbreviations: CRC, colorectal cancer; CUP, cancer of unknown primary; GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma.

CDK12-altered cancer of unknown primary (5.8%) and endometrial cancers (5.0%). For the *CDK12*-altered cases where bi-allelic/mono-allelic status could be reliably determined, we estimated the fraction of cases with bi-allelic alteration (Fig. 1C): the majority of ovarian, breast, prostate, unknown primary, and endometrial cancer cases harbored bi-allelic *CDK12* GAs. Interestingly, in contrast to other cancer types, *CDK12* GAs in breast and gastroesophageal cancers were
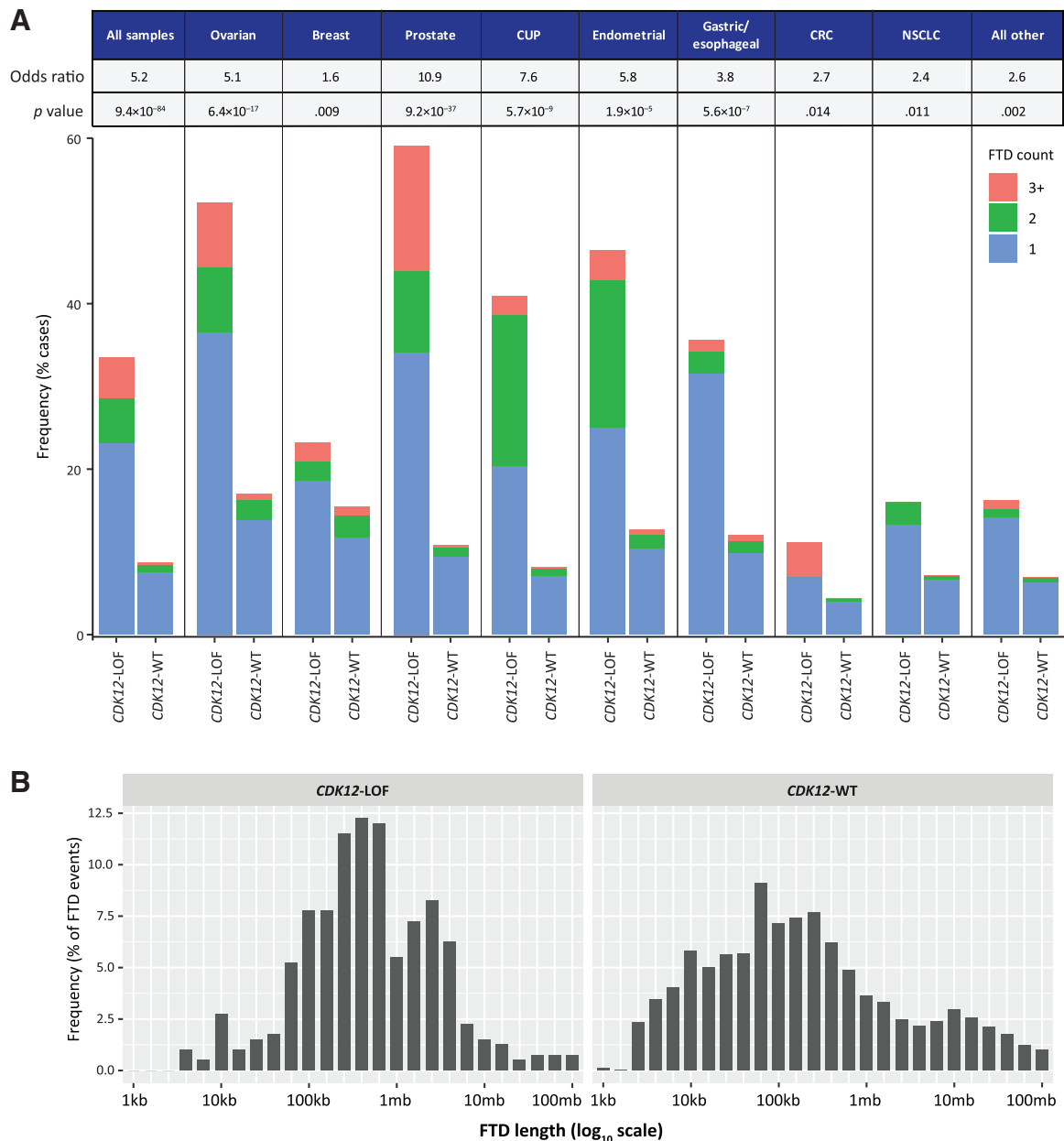
Figure 2. *CDK12* alteration and the tandem-duplicator phenotype (TDP). **(A):** The TDP was quantified by evaluating the number of FTDs detected per case. The distribution of FTD burden was compared for *CDK12*-WT versus *CDK12*-LOF for the overall data set ("all samples"), eight individual tumor types with ≥50 *CDK12* genomic alteration samples, and all other disease types that were grouped together and analyzed as a single group ("all other"). **(B):** Histogram showing the relative size distribution of FTDs identified in *CDK12*-WT and *CDK12*-LOF cases.

Abbreviations: CRC, colorectal cancer; CUP, cancer of unknown primary; FTD, focal tandem duplication; LOS, loss-of-function; NSCLC, non-small cell lung cancer; WT, wild-type.

primarily rearrangements (Fig. 1B; 67.3% and 59.8% of *CDK12*-altered specimens, respectively); however, bi-allelic/mono-allelic status could not generally be determined for such rearrangements.

In prior literature, *CDK12* alterations have been associated with two distinct genomic signatures (TDP and HRD) [1, 3, 7, 9]; therefore, we assessed the association between *CDK12* GAs with these phenotypes by quantifying FTD burden (the number of detected duplicating rearrangements per sample) and percentage gLOH, respectively. Because heterozygous *CDK12* short variant mutations potentially represent passenger events, we focused this analysis on *CDK12* bi-

allelic alterations or rearrangements only (referred to as *CDK12*-LOF alterations). Consistent with previous studies, for ovarian and prostate cancers, FTD burden was significantly increased in samples with *CDK12*-LOF compared with *CDK12* wild-type (*CDK12*-WT) samples (Fig. 2A).

CDK12-LOF was also significantly associated with increased FTD burden across all other cancer types examined (Fig. 2A). The effect size was greatest in prostate cancer, where the mean number of FTDs per tumor was 1.05 for *CDK12*-LOF versus 0.13 for *CDK12*-WT samples (odds ratio [OR] = 10.9, $p = 9.2 \times 10^{-37}$), but it was also high in cancer of unknown primary, endometrial, ovarian, gastroesophageal, and colorectal
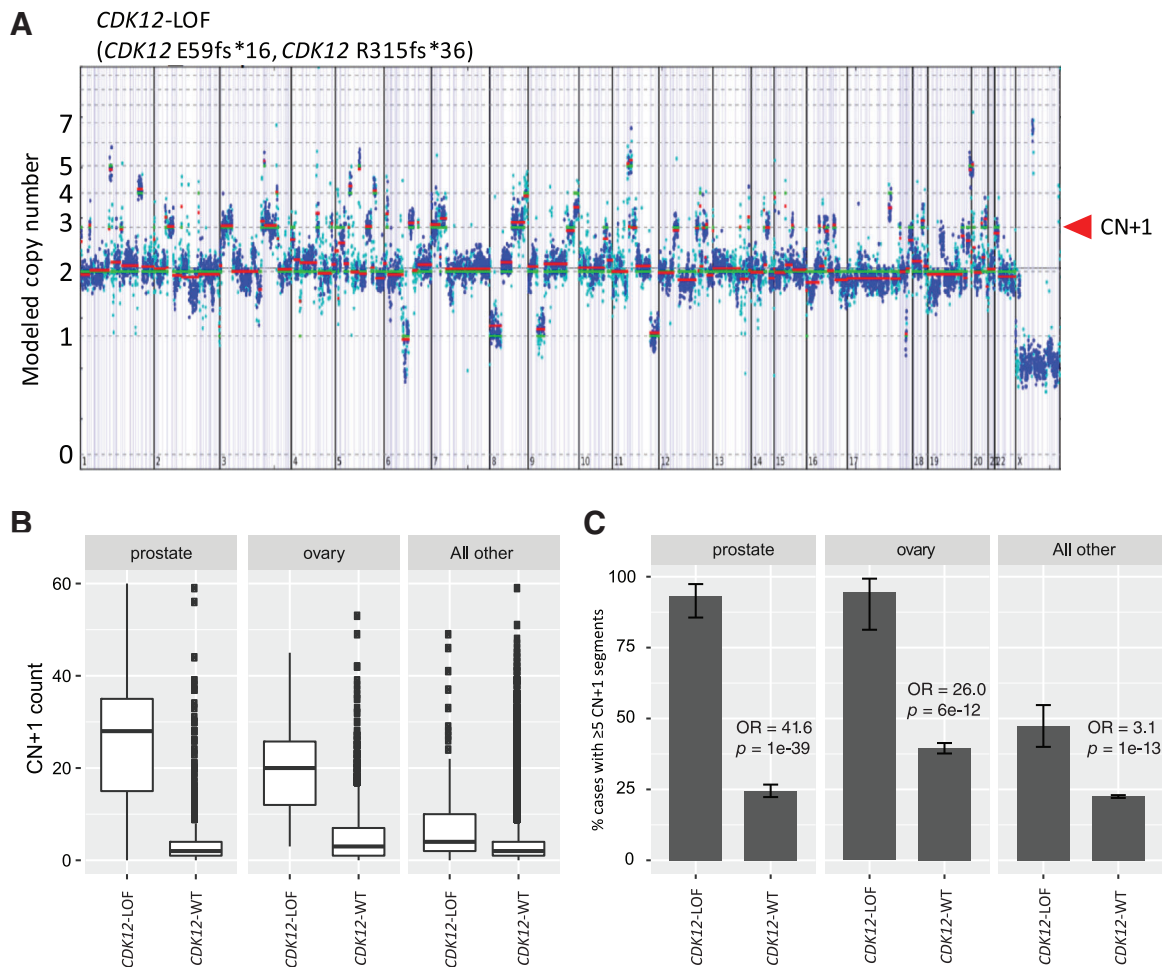
A

CDK12-LOF
(*CDK12* E59fs*16, *CDK12* R315fs*36)

Figure 3. *CDK12* alterations are associated with copy number gains. **(A):** Example of copy number plot from a *CDK12*-LOF prostate tumor harboring multiple *CDK12* truncating mutations. The red arrow indicates short chromosomal segments at copy number 3 (CN + 1), indicative of focal tandem duplications. *x*-axis indicates copy number, *y*-axis indicates genomic position ordered by chromosome number. **(B):** The tandem-duplicator phenotype was quantified by CN + 1 score for *CDK12*-WT versus *CDK12*-LOF cases in ovarian, prostate, and all other cancer types. Box-and-whisker plots: box spans first and third quartiles, the median is denoted by the horizontal line in the box, whiskers indicate maximum and minimum values within 1.5× the interquartile range, black dots indicate outlier events. **(C):** The percentage of cases harboring ≥5 CN + 1 segments was compared for *CDK12*-WT and *CDK12*-LOF cases for prostate, ovarian, and all other cancer types. Error bars indicate 95% confidence interval.

Abbreviations: CN + 1, copy number gain; LOF, loss-of-function; OR, odds ratio; WT, wild-type.

cancers (Fig. 2A; supplemental online Table 4). When considering all *CDK12* GAs including heterozygous mutations, there were also more FTDs observed relative to *CDK12*-WT; however, the magnitude of the association in each cancer type was reduced (supplemental online Table 4). Consistent with prior studies [1, 3, 9, 10], FTDs in cases harboring *CDK12*-LOF alterations had a bimodal size distribution with peaks around 0.4 Mb and 2.5 Mb (Fig. 2B). The fraction of FTDs giving rise to potential gene fusions was similar irrespective of *CDK12* genotype (11% [44/410] for *CDK12*-LOF tumors vs. 12% [1,621/13,847] for *CDK12* wild-type tumors); therefore, the enrichment of FTDs in *CDK12*-LOF cases is also predicted to result in an enrichment in gene fusions that when expressed potentially result in fusion-associated neoantigens [1, 4].

When assessed across all samples, *CDK12*-LOF was associated with HRD (increased gLOH); however, in contrast to the pan-cancer association of *CDK12*-LOF alterations with FTDs, the association of *CDK12*-LOF with HRD was specific to certain cancer types: *CDK12*-LOF was associated with increased

gLOH in non-small cell lung cancer and cancer of unknown primary but not for the other six cancer types assessed (supplemental online Table 5; supplemental online Fig. 2); for all other cancer types without sufficient *CDK12*-LOF samples to be assessed individually, there was an overall association between *CDK12*-LOF and HRD. These results suggest that the role of CDK12 in homologous recombination repair may be tumor type context-dependent.

In ovarian and prostate cancers, *CDK12*-associated FTDs result in a distinct copy number profile with single copy gains dispersed throughout the genome (Fig. 3A) [1, 3, 9]. In contrast to FTDs that are detected at precise rearrangement breakpoints, copy gains are measurable over the entire duplicated segment and therefore provide a further opportunity to quantify the TDP. As a surrogate measure of FTDs, we generated a CN + 1 score by evaluating the number of segments (between 10 kb and 10 Mb) with single copy gains greater than the ploidy of the sample. We assessed the association of *CDK12*-LOF genotype with CN + 1 scores in ovarian
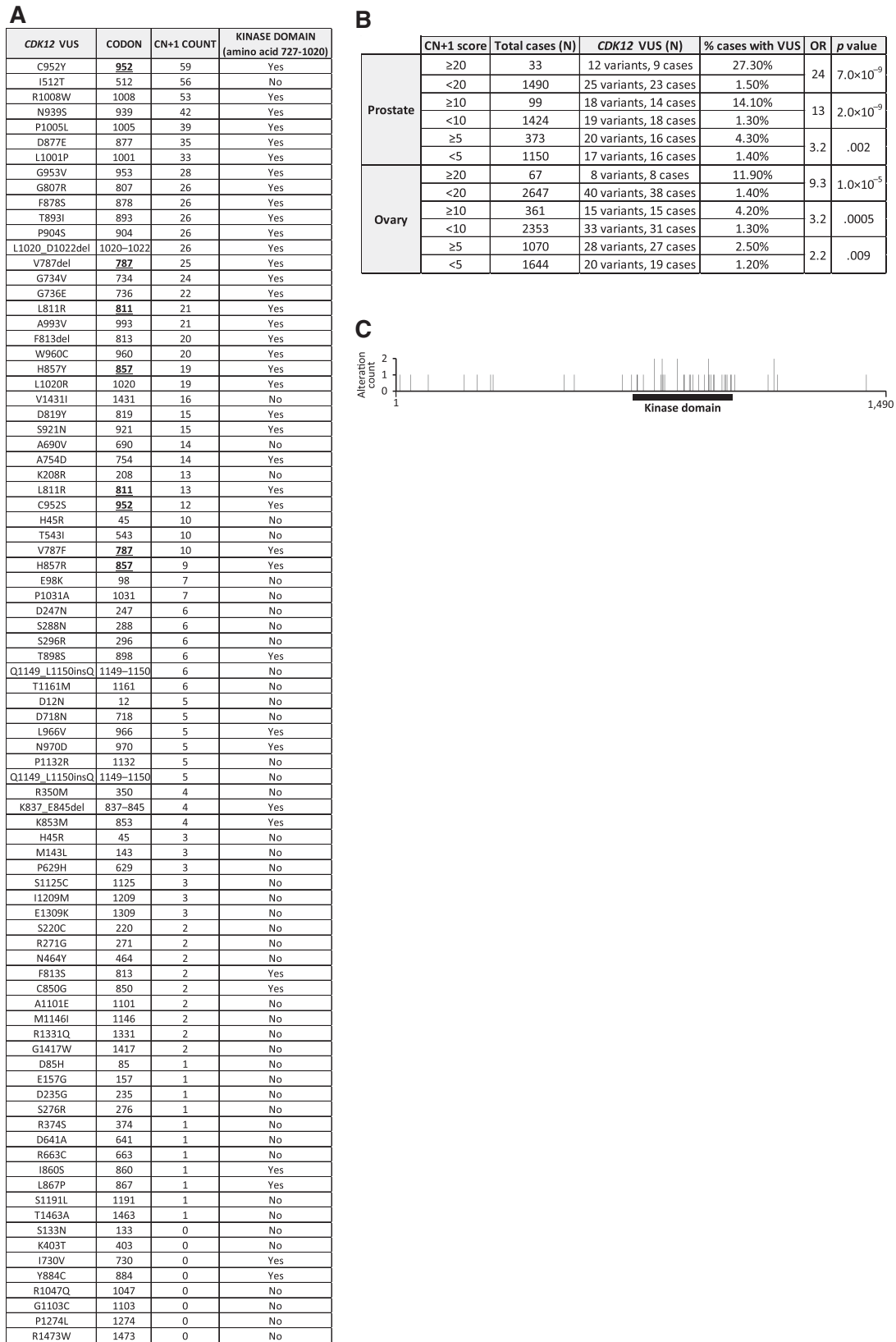
A

CDK12 VUS	CODON	CN+1 COUNT	KINASE DOMAIN (amino acid 727-1020)
C952Y	**952**	59	Yes
I512T	512	56	No
R1008W	1008	53	Yes
N939S	939	42	Yes
P1005L	1005	39	Yes
D877E	877	35	Yes
L1001P	1001	33	Yes
G953V	953	28	Yes
G807R	807	26	Yes
F878S	878	26	Yes
T893I	893	26	Yes
P904S	904	26	Yes
L1020_D1022del	1020–1022	26	Yes
V787del	**787**	25	Yes
G734V	734	24	Yes
G736E	736	22	Yes
L811R	**811**	21	Yes
A993V	993	21	Yes
F813del	813	20	Yes
W960C	960	20	Yes
H857Y	**857**	19	Yes
L1020R	1020	19	Yes
V1431I	1431	16	No
D819Y	819	15	Yes
S921N	921	15	Yes
A690V	690	14	No
A754D	754	14	Yes
K208R	208	13	No
L811R	**811**	13	Yes
C952S	**952**	12	Yes
H45R	45	10	No
T543I	543	10	No
V787F	**787**	10	Yes
H857R	**857**	9	Yes
E98K	98	7	No
P1031A	1031	7	No
D247N	247	6	No
S288N	288	6	No
S296R	296	6	No
T898S	898	6	Yes
Q1149_L1150insQ	1149–1150	6	No
T1161M	1161	6	No
D12N	12	5	No
D718N	718	5	No
L966V	966	5	Yes
N970D	970	5	Yes
P1132R	1132	5	No
Q1149_L1150insQ	1149–1150	5	No
R350M	350	4	No
K837_E845del	837–845	4	Yes
K853M	853	4	Yes
H45R	45	3	No
M143L	143	3	No
P629H	629	3	No
S1125C	1125	3	No
I1209M	1209	3	No
E1309K	1309	3	No
S220C	220	2	No
R271G	271	2	No
N464Y	464	2	No
F813S	813	2	Yes
C850G	850	2	Yes
A1101E	1101	2	No
M1146I	1146	2	No
R1331Q	1331	2	No
G1417W	1417	2	No
D85H	85	1	No
E157G	157	1	No
D235G	235	1	No
S276R	276	1	No
R374S	374	1	No
D641A	641	1	No
R663C	663	1	No
I860S	860	1	Yes
L867P	867	1	Yes
S1191L	1191	1	No
T1463A	1463	1	No
S133N	133	0	No
K403T	403	0	No
I730V	730	0	Yes
Y884C	884	0	Yes
R1047Q	1047	0	No
G1103C	1103	0	No
P1274L	1274	0	No
R1473W	1473	0	No

B

	CN+1 score	Total cases (N)	CDK12 VUS (N)	% cases with VUS	OR	p value
Prostate	≥20	33	12 variants, 9 cases	27.30%	24	7.0×10⁻⁹
	<20	1490	25 variants, 23 cases	1.50%		
	≥10	99	18 variants, 14 cases	14.10%	13	2.0×10⁻⁹
	<10	1424	19 variants, 18 cases	1.30%		
	≥5	373	20 variants, 16 cases	4.30%	3.2	.002
	<5	1150	17 variants, 16 cases	1.40%		
Ovary	≥20	67	8 variants, 8 cases	11.90%	9.3	1.0×10⁻⁵
	<20	2647	40 variants, 38 cases	1.40%		
	≥10	361	15 variants, 15 cases	4.20%	3.2	.0005
	<10	2353	33 variants, 31 cases	1.30%		
	≥5	1070	28 variants, 27 cases	2.50%	2.2	.009
	<5	1644	20 variants, 19 cases	1.20%		

C

Figure 4. CDK12 variants of unknown significance (VUS). **(A):** Details of CDK12 VUS alterations identified in CDK12-wild-type (WT) prostate and ovarian cancer cases and the number of CN + 1 segments identified in each case. **(B):** CDK12-WT cases at different CN + 1 thresholds were evaluated for the frequency at which CDK12 VUS alterations were identified. **(C):** CDK12 VUS alterations identified in CDK12-WT cases with ≥5 CN + 1 segments and their location relative to the kinase domain.
Abbreviations: CN + 1, copy number gain; OR, odds ratio; VUS, variants of unknown significance.

and prostate cancers. *CDK12*-LOF cases had significantly higher CN + 1 scores compared with *CDK12*-WT samples (Fig. 3B; supplemental online Table 6). The fraction of cases with ≥5 CN + 1 segments was increased in *CDK12*-LOF tumors for prostate cancer (OR = 41.6, $p = 10^{-39}$) and ovarian cancer (OR = 26.0, $p = 10^{-12}$) and also significantly increased in *CDK12*-LOF cases for all other non-prostate/ovarian cancer types, albeit with reduced magnitude (OR = 3.1, $p = 10^{-13}$; Fig. 3C; supplemental online Table 6).

We then asked whether there were any distinctive features associated with *CDK12*-WT cases harboring high CN + 1 scores. We evaluated prostate and ovarian tumors where the association with CN + 1 score and *CDK12* status was strongest, and in doing so, we found that *CDK12* VUS were significantly enriched in *CDK12*-WT cases with high CN + 1 scores versus those with low CN + 1 scores (Fig. 4A, 4B). The majority of the *CDK12* VUS mutations in high CN + 1 cases were located in the kinase domain (31/48, 65%); in contrast, fewer *CDK12* kinase domain VUS were found in low CN + 1 cases (8/37, 22%; Fig. 4A, 4C). Although these *CDK12* VUS alterations that were associated with high CN + 1 scores have not been previously been functionally characterized as deleterious mutations or observed as somatic mutations in cancer, mutated residues included those involved in kinase activation (H857Y/R, F878S, T893I) or interactions between CDK12 and its heterodimeric cyclin K partner (G807R), as well as recurrently mutated residues, suggesting functional importance (Fig. 4) [15].

DISCUSSION

The recent tumor-agnostic approval of ICI agents targeting the programmed cell death 1 (PD-1) checkpoint for patients with mismatch repair deficiency and/or microsatellite instability has demonstrated the clinical utility of biomarker strategies based on high predicted neoantigen burden to select patients likely to derive benefit from ICI. *CDK12*-LOF is associated with expressed fusion-induced neoantigens derived from FTDs and has therefore emerged as a potential candidate predictive biomarker for ICI sensitivity in prostate cancer [1, 4]. However, *CDK12* status and its association with FTDs has not previously been studied beyond ovarian and prostate cancer.

To assess the pan-cancer landscape of *CDK12*-LOF genomic alterations and to evaluate whether the association with FTD extends broadly across tumor types, we assessed our database of 142,133 consecutive tumor samples that were sequenced using a clinical-grade CGP assay. Although *CDK12* alterations have been reported in The Cancer Genome Atlas (TCGA) [16], the specimens evaluated in this study were from routine clinical testing enriched in patients with advanced disease [17, 18]; these results therefore represent the landscape of *CDK12* alteration in the real-world setting that may be distinct from unselected primary tumors. We identify *CDK12* GA in 1.1% of all cancers; although the overall prevalence of *CDK12* GAs is relatively low, consistent with an analysis of *CDK12* alteration prevalence in the TCGA [16], specific cancer types including gastroesophageal, breast, and endometrial harbored *CDK12* GAs at a > 1% frequency. Notably, NGS-based screening strategies have enabled successful completion of

basket trials targeting *NTRK* alterations that also occur at low frequencies [19], recently leading to the approval of larotrectinib for *NTRK* fusion-positive cancers of any histology.

Our understanding of the functional role of CDK12 is evolving. It was originally thought that CDK12 may be a mediator of homologous recombination DNA repair, and that *CDK12*-LOF mutations would result in homologous recombination deficiency [7]. However, consistent with more recent data from human ovarian and prostate cancers [1, 9], we found no association between *CDK12*-LOF alterations and the HRD phenotype (as measured by gLOH) in prostate and ovarian cancers, and only marginal associations in other cancer types evaluated. This finding is in keeping with emerging clinical data suggesting that PARP inhibitors are largely ineffective in prostate cancers with CDK12 deficiency [8]. Therefore, the role of CDK12 in homologous recombination repair and the role of *CDK12* alteration as a biomarker for PARP inhibitors might be limited to specific tumor types. To this end, our results demonstrating the association between *CDK12*-LOF and the TDP across tumor types suggests immune checkpoint blockade may be a more rational approach that may be broadly applicable for the treatment of patients with *CDK12*-altered cancers, and such basket trials evaluating *CDK12* as a biomarker for ICI are currently underway (NCT03570619).

A limitation of this study is that we employed a targeted NGS assay (covering 1.1 Mb of sequenced DNA) rather than whole-exome sequencing (WES) or whole-genome sequencing (WGS); therefore; FTDs that may have occurred outside of the captured genomic regions would not be detected, resulting in limited sensitivity to robustly capture the *CDK12*-associated FTD phenotype. Nevertheless, we were able to recapitulate the observation of *CDK12*-associated FTD described in prior WGS or WES studies of ovarian and prostate cancers [1, 3, 9], and, using a much larger genomic data set of cancer samples, we further demonstrated that *CDK12*-LOF is broadly associated with FTDs in many diverse tumor types; the association of *CDK12* with FTDs in cancer types other than ovarian and prostate cancer has not been extensively evaluated in previous studies of *CDK12* in the TCGA data sets [10, 16]. An additional limitation was our inability to interrogate genomic signatures in *CDK12*-altered cancer types with fewer than 50 cases of *CDK12* inactivation, therefore restricting cancer type-specific analyses to only eight tumor types; however, *CDK12*-LOF were also associated with FTDs in other tumor types overall (Fig. 2A). Finally, because we were unable to resolve the exact genomic coordinates of the FTD breakpoints, we were unable to assess actual or predicted gene-fusion antigenicity or to estimate FINA burden.

CDK12 is emerging as a candidate genomic biomarker for ICI therapy, and potential biomarker strategies incorporating quantitative assessment of the *CDK12*-associated TDP could provide additional information beyond *CDK12* genotype alone. In contrast to WGS or WES that are usually employed in the research setting, targeted NGS is more routinely available as a tool in clinical practice; therefore, the ability to quantify *CDK12*-associated TDP using targeted NGS may be required for clinical decision making. As an analogy, tumor mutational burden as determined by targeted NGS has been correlated with results from WES and is increasingly being

used in the clinical arena [20]. In ovarian and prostate cancers in particular, quantitative measurement of the TDP using the CN + 1 score enabled further resolution of the FTD signature and also allowed post hoc identification of several recurrent kinase-domain *CDK12*-VUS alterations that are likely pathogenic. In other tumor types, CN + 1 score was not generally associated with *CDK12* status, and further studies may be required to refine the CN + 1 score in non-ovarian/prostate cancer histologies.

CONCLUSION

We report the first pan-cancer analysis of *CDK12* alterations across multiple tumor types, showing a modest prevalence (>1%) of this genetic alteration in at least 11 cancer types and association of *CDK12*-LOF with increased FTD burden across all tumor types examined. We suggest that *CDK12* warrants broad evaluation as a candidate genomic biomarker for ICI benefit, and that FTD signatures may further refine the predictive value of this potential marker.

AUTHOR CONTRIBUTIONS

Conception/design: Ethan S. Sokol, Jon H. Chung, Emmanuel S. Antonarakis

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Collection and/or assembly of data: Ethan S. Sokol, Dean Pavlick, Garrett M. Frampton, Jeffrey S. Ross, Vincent A. Miller, Siraj M. Ali, Tamara L. Lotan, Drew M. Pardoll, Jon H. Chung, Emmanuel S. Antonarakis

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DISCLOSURES

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