Pan-cancer landscape of *CD274* (PD-L1) copy number changes in 244 584 patient samples and the correlation with PD-L1 protein expression

Richard S.P. Huang ¹, Karthikeyan Murugesan,¹ Meagan Monteson,¹ Dean C. Pavlick,¹ Douglas A. Mata,¹ Matthew C. Hiemenz,¹ Brennan Decker,¹ Garrett Frampton,¹ Lee A. Albacker,¹ Jeffrey S. Ross^{1,2}

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¹Foundation Medicine Inc, Cambridge, Massachusetts, USA

²Department of Pathology, State University of New York (SUNY) Upstate Medical University, Syracuse, New York, USA

Correspondence to

Dr Richard S.P. Huang;
rhuang@foundationmedicine.com

ABSTRACT

Introduction Several studies have shown clinical outcomes data that support the use of *CD274* (PD-L1) copy-number (CN) gains and/or losses as a biomarker for immune checkpoint inhibitor (ICPI). Here, we present the landscape of *CD274* CN changes across a large cohort of solid tumor cases and correlate these with PD-L1 protein expression by immunohistochemistry.

Methods We analyzed all cases that underwent comprehensive genomic profiling (CGP) testing at Foundation Medicine between August 2014 and June 2020. *CD274* CN changes were correlated with PD-L1 expression in tumor types where there were Food and Drug Administration approved companion diagnostic (CDx) claims and the CDx assay was used to assess PD-L1 expression.

Results In all, 244 584 samples representing 290 solid tumor types were included in the study. Overall, 17.6% (42 983/244 584) had *CD274* CN gains (>specimen ploidy), 44.6% (108 970/244 584) were *CD274* CN neutral, and 37.9% (92 631/244 584) had *CD274* CN loss. Using different CN cut offs to define *CD274* positivity resulted in different prevalence estimates: ploidy +1, 17.4% (42 636/244 584); ploidy +2, 6.2% (15 183/244 584); ploidy +3, 2.2% (5375/244 584); ploidy +4, 1.1% (2712/244 584); and ploidy +8, 0.2% (434/244 584). The prevalence of CN changes and CN positivity varied based on tumor type. *CD274* CN gains were significantly associated with PD-L1 positivity in NSCLC, urothelial carcinoma, breast carcinoma, cervical carcinoma, esophagus squamous cell carcinoma (SCC) and head and neck SCC (ORs 3.3, 3.0, 2.0, 4.5, 3.8, 8.4, 1.4, respectively; $p < 0.05$) and with microsatellite instability status in only clinically relevant tumor types (gastric adenocarcinoma, colorectal adenocarcinoma, uterine endometrial adenocarcinoma, esophageal adenocarcinoma and gastroesophageal junction adenocarcinoma (OR: 5.2, 1.9, 3.2, 3.7 and 6.5, respectively; $p < 0.05$)). Conversely, *CD274* CN changes were not significantly correlated with tumor mutational burden in almost all the tumor types.

Conclusion *CD274* CN changes and PD-L1 expression were highly correlated in multiple tumor types. These prevalence data on *CD274* CN changes across a large cohort of different solid tumors can be used to design future clinical studies to assess whether *CD274* CN changes could be a potential biomarker for ICPI.

INTRODUCTION

Immune checkpoint inhibitors (ICPI) have revolutionized treatment options for cancer patients, and three biomarkers are currently approved by the United States Food and Drug Administration (FDA) as companion diagnostics (CDx) for ICPI.¹ These include microsatellite instability (MSI) testing, where MSI-High (MSI-H) patients with solid tumors are eligible for pembrolizumab; tumor mutational burden (TMB) testing by comprehensive genomic profiling (CGP), where solid tumor patients with TMB ≥ 10 mutations/megabase (mut/Mb) (TMB-High, TMB-H) are also eligible for pembrolizumab; and PD-L1 expression measured by immunohistochemistry (IHC), where PD-L1 positive tumor cells or immunocytes in certain tumor types enable the selection of ICPI such as pembrolizumab, atezolizumab or nivolumab.^{2–6} One promising but not as well studied biomarker for ICPI is *CD274* (PD-L1) gene copy number (CN) changes.

In clinically advanced Hodgkin lymphoma, a tumor type that generally responds well to ICPI, *CD274* CN gains is almost always present.^{7–9} In addition, in a study using CGP, *CD274* amplification (defined as ploidy +4 (CN 6)) was identified in 0.7% of patients across a large cohort of diverse solid tumors, and some evidence emerged indicating that *CD274* amplification is a predictor of ICPI response.¹⁰ More recently, in the SAFIR02-IMMUNO Randomized Phase II Trial, exploratory analysis has shown that *CD274* amplification (defined as CN ≥ 5) and CN gains (CN 3–4) were predictors of durvalumab response in metastatic breast cancer.¹¹ In non-small-cell lung cancer (NSCLC), Lamberti *et al.*¹² discovered that *CD274* loss was associated with a lower response rate and progression

free survival when compared with patients without *CD274* loss. In another study, the authors concluded that *CD274* amplification (defined as *CD274* to CEP9 ratio of at least 2.0 as determined by fluorescence in situ hybridization) was associated with response to nivolumab monotherapy in NSCLC patients.¹³ While these studies used different methodologies to detect *CD274* CN changes, overall the data showed that increases in *CD274* CN were associated with better response to ICPI and decreases in *CD274* CN were associated with an attenuated response to ICPI.

Currently, evidence exists in the literature supporting *CD274* CN gains and losses as a promising biomarker for ICPI; however, the landscape of *CD274* CN changes in different tumor types has not been well described. In addition, while a strong correlation was observed in *CD274* amplification (ploidy +4 (CN 6) with CGP) and PD-L1 positivity across solid tumor types, a more nuanced study of *CD274* CN and PD-L1 expression is lacking.^{3,12} Here, we investigated the landscape of *CD274* CN changes as detected by CGP in a large cohort of diverse solid tumor cases and correlated the *CD274* CN to the well-established ICPI biomarkers of PD-L1 IHC, MSI and TMB. We show that the prevalence of *CD274* CN changes varied based on tumor type, and that *CD274* CN changes and PD-L1 expression were highly correlated in multiple tumor types.

MATERIALS AND METHODS

Data collection

We analyzed all cases that underwent CGP testing at Foundation Medicine between August 2014 and June 2020. Available clinical information for the patient samples were extracted from accompanying test requisition form and pathology reports.

All specimens were assigned a diagnosis by a board-certified pathologist based on microscopic examination of a H&E-stained slide from the formalin-fixed-paraffin-embedded (FFPE) tissue, pathology report, and clinical information provided by the ordering physician.

Comprehensive genomic profiling

CGP was performed on hybridization-captured, adaptor ligation-based libraries using DNA extracted from FFPE tumor tissue in a Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists (CAP)-accredited laboratory (Foundation Medicine, Cambridge, Massachusetts, USA). The samples were sequenced for up to 324 cancer related genes and/or select gene rearrangements.¹⁴

CN alterations were detected using a comparative genomic hybridization-like method applied to next generation sequencing data.^{14,15} In the laboratory, each specimen was analyzed alongside a process-matched normal control (an internally validated mixture of 10 heterozygous diploid samples from the HapMap project), with custom algorithms to normalize the sequence coverage distribution across captured DNA regions.

Log-ratios of normalized coverage data for exonic, intronic, and SNP targets accounting for stromal admixture, as well as genome-wide SNP frequencies, were used to generate the profiles. Using circular binary segmentation, custom algorithms further clustered groups of targets and SNP frequencies to define upper and lower bounds of genomic segments. Empirical Bayesian algorithms employed a distribution of parameters including purity and base ploidy and probability matrices were derived using different statistical sampling methodologies to fit these data. Specimen level ploidy was estimated as described by Sun *et al*.¹⁵ Computational models were reviewed by expert analysts for each sample.¹⁴ *CD274* CN gain was defined as any CN > ploidy of the specimen, *CD274* CN neutral was any CN that equaled the ploidy of the specimen, and *CD274* CN loss was any CN < ploidy of the specimen. We also explored prevalence rates of different CN cut offs (ploidy +1 (CN 3), ploidy +2 (CN 4), ploidy +3 (CN 5), ploidy +4 (CN 6), and ploidy +8 (CN 10)) to define *CD274* CN positivity.

TMB was determined on 0.8–1.1 Mb of sequenced DNA and calculated as the number of non-driver somatic coding mut/Mb of genome sequenced.¹⁶ For TMB, we considered a TMB cut-off of at least 10 mut/Mb as TMB-H in our analysis based on the FDA pan-solid tumor CDx approval for pembrolizumab.¹⁷ MSI status was determined by analyzing 114 intronic homopolymer repeat loci for length variability, as previously described.¹⁸ MSI positivity was defined as MSI-H as per the pan-tumor approval for pembrolizumab.¹⁹

As an exploratory analysis, we hypothesized that the high rates of *CD274* CN gains in cervix squamous cell carcinoma (SCC) and Head and Neck (HN) SCC could be due to human papillomavirus (HPV) infection, and therefore explored the HPV status of these patients. To determine the HPV status of the samples, we performed de novo assembly of non-human sequencing reads and BLASTn comparison against all viral nucleotide sequences in the NCBI RefSeq database were used to detect the presence of HPV genome sequences (Research Use Only).

PD-L1 IHC

PD-L1 IHC testing was run and interpreted by board-certified pathologists according to the manufacturer instructions in a CLIA-certified and CAP-accredited laboratory (Foundation Medicine, Morrisville, North Carolina) for a subset of specimens in this cohort.^{20,21} Specially, we examined the tumor types with a PD-L1 CDx approval: DAKO 22C3 PD-L1 assay for NSCLC (tumor proportion score cut-off ≥1), cervical carcinoma (combined positive score (CPS) cut-off ≥1), head and neck SCC (CPS cut-off ≥1), gastric/gastroesophageal adenocarcinoma (CPS cut-off ≥1), urothelial carcinoma (CPS cut-off ≥10), and esophageal SCC (CPS cut-off ≥10); and VENTANA SP142 PD-L1 assay for breast carcinoma at tumor infiltrating immune cell cut-off of 1%.^{22–24}

Analyses

Statistical analyses were performed using R V.3.6.0 (R Foundation for Statistical Computing, Vienna, Austria) and Python V.2.7.16.^{25 26} Differences among categorical variables were assessed using the Fisher's exact test. Statistical tests were two sided and multiple hypothesis testing correction was performed using the Benjamini-Hochberg procedure.

Currently, PD-L1 IHC is the gold standard for the tumor types with a PD-L1 CDx claim. We further sought to investigate the sensitivity, specificity, positive predictive value and negative predictive value of *CD274* CN positivity (based on different CN cut-offs) when compared with PD-L1 IHC positivity.

RESULTS

CD274 CN changes in various tumor types

We analyzed *CD274* CN in 244,584 solid tumor samples representing 290 solid tumor types. Overall, 17.6% (42,983/244,584) had *CD274* CN gains (CN >ploidy), 44.6% (108,970/244,584) were *CD274* CN neutral (CN=ploidy), and 37.9% (92,631/244,584) had *CD274* CN loss (CN <ploidy) (online supplemental table 1).

Among tumor types with ≥1000 samples, cervical SCC (31.3%), lung small-cell undifferentiated carcinoma (27.0%), and head and neck SCC (HNSCC) (25.7%) had the highest frequencies of *CD274* CN gain (figure 1). Conversely, skin melanoma (8.1%), pancreatic ductal adenocarcinoma (8.5%), and glioblastoma (9.3%) had the lowest prevalence of *CD274* gain. In addition, in the cohort with *CD274* CN gains, we observed the highest magnitude of CN gains in tumor types with SCC morphology (figure 2). A positive correlation of *CD274* CN gains with HPV infection in cervical SCC and HNSCC was observed (OR=1.4, $p=0.17$; OR 3.8, $p=8.8\times10^{-31}$). Pancreatic ductal adenocarcinoma (56.3%), gallbladder adenocarcinoma (55.5%), and cutaneous melanoma (55.4%) had the highest frequencies of *CD274* CN loss. This contrasted with cervical SCC (14.4%), appendiceal adenocarcinoma (16.4%), and uterine endometrial adenocarcinoma (16.6%), which had the lowest frequencies of *CD274* CN loss (figure 1).

In addition, we explored the prevalence of *CD274* CN positivity based on different CN cut offs. When using ploidy +1 (CN 3) as the cut-off, the overall positivity was 17.4% (42,636/244,584); when using ploidy +2 (CN 4) as the cut-off, the overall positivity was 6.2% (15,183/244,584); when using ploidy +3 (CN 5) as the cut-off, the overall positivity was 2.2% (5375/244 584); when using ploidy +4 (CN 6) as the cut-off, the overall positivity was 1.1% (2712/244 584); and when using ploidy +8 (CN 10) as the cut-off, the overall positivity was 0.2% (434/244 584). *CD274* CN positivity in different tumor types also varied using these different cut-offs (figure 3 and online supplemental table 2).

CD274 CN correlation with PD-L1 IHC

CD274 CN gains were highly correlated with PD-L1 IHC positive status in almost all tumor types where a CDx

assay was available (figure 4A). Interestingly, HNSCC had the highest OR in this comparison and gastric/esophageal adenocarcinoma which had the lowest OR (8.44, $p=3.1\times10^{-2}$; 1.41, $p=8.7\times10^{-2}$; respectively). NSCLC, urothelial carcinoma, breast carcinoma, cervical carcinoma, and esophageal SCC all had a positive and significant OR (3.29, $p=3.2\times10^{-173}$; 2.97, $p=3.2\times10^{-15}$; 1.96, $p=1.6\times10^{-13}$; 4.51, $p=2.1\times10^{-5}$; and 3.81, $p=8.7\times10^{-2}$, respectively).

While *CD274* CN changes were highly correlated with PD-L1 IHC status across multiple tumor types, at a population level, there was still a subset of patients in which they were not. Specifically, 4.6% (1378/29887) of the overall cohort with *CD274* CN and PD-L1 IHC data had *CD274* gain but were PD-L1 negative (table 1). Conversely, 23.3% (6953/29 887) had *CD274* loss but were PD-L1 IHC positive (table 1).

When compared with PD-L1 IHC, *CD274* CN positivity (at different CN cut-offs) is highly specific and has high positive predictive value (figure 4B and online supplemental table 3). On the other hand, sensitivity and the negative predictive value is lower. Importantly, the sensitivity, specificity, positive predictive value, and negative predictive value varied depending on which cut-off we used to define *CD274* CN positivity and varied depending on tumor type/PD-L1 CDx assay and scoring algorithm used (figure 4B and online supplemental table 3).

CD274 CN correlation with TMB and MSI

CD274 CN gains were not significantly correlated with TMB-H in almost all (98.6%, 286/290) tumor types. *CD274* CN gain were significantly correlated with TMB in only four tumor types: lung adenocarcinoma, gastric adenocarcinoma, uterine endometrial adenocarcinoma, and bladder urothelial carcinoma (OR: 1.2, $p=9.3\times10^{-6}$; 2.3, $p=1.6\times10^{-5}$; 2.3, $p=4.7\times10^{-5}$; and 1.4, $p=4.6\times10^{-2}$, respectively) (figure 5A).

In the tumor types in which MSI is most clinically relevant (gastric adenocarcinoma, colorectal adenocarcinoma, uterine endometrial adenocarcinoma, esophageal adenocarcinoma, and gastroesophageal junction adenocarcinoma), we observed a significant correlation between *CD274* CN gains and MSI-H status (OR: 5.2, $p=4.9\times10^{-10}$; 1.9, $p=6.7\times10^{-8}$; 3.2, $p=2.1\times10^{-6}$; 3.7, $p=5.5\times10^{-3}$; and 6.5, $p=2.3\times10^{-2}$, respectively) (figure 5B). Most of the remaining tumor types did not have significant correlation between *CD274* CN gains and MSI-H.

DISCUSSION

In this study, we present prevalence data on *CD274* CN losses, gains, and positivity (defined by different *CD274* CN cut-offs) in over 240 000 patient samples across 290 solid tumor types. While Goodman *et al*¹⁰ previously presented data on *CD274* amplification status (defined as ploidy +4) on a large cohort of patients, recent clinical data suggests that CN loss, CN gain, and amplification based on different cut offs can represent both negative

Figure 1 Prevalence of *CD274* copy number (CN) gains and losses in different tumor types. Cervical SCC (31.3%), lung small-cell undifferentiated carcinoma (27.0%), and head and neck SCC (25.7%) had the highest frequencies of *CD274* CN gain; cutaneous melanoma (8.1%), pancreatic ductal adenocarcinoma (8.5%) and glioblastoma (9.3%) had the lowest frequencies of *CD274* gain. Pancreatic ductal adenocarcinoma (56.3%), gallbladder adenocarcinoma (55.5%) and cutaneous melanoma (55.4%) had the highest frequencies of *CD274* loss; cervical SCC (14.4%), appendiceal adenocarcinoma (16.4%) and uterine endometrial adenocarcinoma (16.6%) had the lowest frequencies of *CD274* loss. Only tumor types with at least 1000 samples are shown.

Figure 2 Histogram of degree of *CD274* CN gains in different tumor types. The highest levels of CN gains were observed in tumor types with SCC morphology. Only tumor types with at least 1000 samples are shown. CN, copy number.

Figure 3 Histogram showing the prevalence of *CD274* copy number (CN) positivity at different CN cut-offs in different tumor types. Specially, CN cut-off at ploidy +1 (CN 3), ploidy +2 (CN 4), and ploidy +3 (CN 5) are shown here. Only tumor types with at least 1000 samples are shown.

A

Disease	Odds Ratio	P Value
NSCLC	3.29	3.2×10^{-173}
Urothelial Carcinoma	2.97	3.2×10^{-15}
Breast Carcinoma	1.96	1.6×10^{-13}
Cervical Carcinoma	4.51	2.1×10^{-5}
Esophageal SCC	3.81	1.2×10^{-3}
HNSCC	8.44	3.1×10^{-2}
Gastric/Esophageal Adenocarcinoma	1.41	8.7×10^{-2}

B

Figure 4 (A) OR forest plot of *CD274* copy number (CN) gains and PD-L1 immunohistochemistry (IHC) positivity. *CD274* CN gains were highly correlated with PD-L1 IHC positivity in almost all tumor types where a companion diagnostic assay was available. (B) Sensitivity, specificity, positive predictive value, and negative predictive value of *CD274* CN positivity (defined at different cut-offs) when compared with PD-L1 IHC positivity. When compared with PD-L1 IHC, *CD274* CN positivity is highly specific and has high positive predictive value. On the other hand, sensitivity and the negative predictive value is lower. Importantly, the sensitivity, specificity, positive predictive value, and negative predictive value varied depending on which cut-off we used to define *CD274* CN positivity and varied depending on tumor type/PD-L1 CDx assay and scoring algorithm used. CN 3=ploidy +1, CN 4=ploidy +2, CN 5=ploidy +3, and so forth. HNSCC, head and neck SCC; NSCLC, non-small-cell lung cancer.

Table 1 Subset of patients in which CD274 CN gains/losses did not correlate with PD-L1 IHC positivity				
Diagnosis	CD274 CN gain with PD-L1 negative (%)	N	CD274 CN loss with PD-L1 positive (%)	N
NSCLC (n=20 755)	3.9	813	24.7	5125
Urothelial carcinoma (n=1742)	6.8	118	15.6	272
Breast carcinoma (n=3833)	10.0	384	12.7	486
Cervix carcinoma (n=701)	1.9	13	13.3	93
Esophagus SCC (n=208)	4.8	10	22.1	46
HNSCC (n=655)	0.2	1	31.0	203
Gastric/esophageal adenocarcinoma (n=1993)	2.0	39	36.5	728

HNSCC, head and neck SCC; IHC, immunohistochemistry; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma.

Figure 5 (A) volcano plot depicting the association of *CD274* copy number (CN) gains/losses with tumor mutational burden (TMB). The two-tailed Fisher’s exact test was used to evaluate the p values and ORs, to determine associations between *CD274* CN gains/losses and TMB category (TMB-H or TMB-L) for every tumor type. The Benjamini Hochberg procedure was used to estimate the adjusted p values. Significant correlations were observed in four tumor types: lung adenocarcinoma, gastric adenocarcinoma, uterine endometrial adenocarcinoma, and bladder urothelial carcinoma. (B) Volcano plot of *CD274* CN gain/losses with microsatellite instability (MSI) status. The two-tailed Fisher’s exact test was used to evaluate the p values and ORs, to determine associations between *CD274* CN change and MSI category (MSI-H or microsatellite stable [MSS]), for every tumor type. The Benjamini Hochberg procedure was used to estimate the adjusted p values. In the clinically relevant MSI tumor types (gastric adenocarcinoma, colorectal adenocarcinoma, uterine endometrial adenocarcinoma, esophageal adenocarcinoma, and gastroesophageal junction adenocarcinoma), we observe a significant correlation between *CD274* CN and MSI. For both A and B, only tumor types with at least 1000 samples are shown and the horizontal red dotted line represents an adjusted p value of 0.05 and the vertical dotted line represent an OR of one. MSI-H, MSI-high; TMB-H, TMB-high; TMB-L, TMB-low.

and positive predictive biomarkers for ICPI response.^{11–13} In this study, 17.4% had *CD274* CN ≥ploidy +1 (CN 3), 6.2% had *CD274* CN ≥ploidy +2 (CN 4), and 2.2% had

CD274 CN $\geq$ ploidy +3 (CN 5) across tumor types, which is 25-fold, 9-fold, and 3-fold higher, respectively, than when using *CD274* CN $\geq$ ploidy +4 (CN 6), where Goodman *et al*¹⁰ only found 0.7% of solid tumors in their cohort as amplified. Of note, in our current cohort, we found that 1.1% (2712/244 584) had a CN $\geq$ ploidy +4 (CN 6), which is higher than the 0.7% (843/118 187) that Goodman *et al*¹⁰ described. The prevalence in our study likely more accurately describes the real-world prevalence since our data set has twice the number of samples when compared with the previous study. Future clinical trials with outcomes data are needed to assess the optimal *CD274* CN cut-off for a patient to be considered positive and whether *CD274* CN loss is a negative predictor for ICPI response in all tumor types or only certain tumor types. Given the varied levels of CN changes in the various tumor types presented in this study, we suspect that the CN cut offs that are correlated with ICPI response will vary based on tumor type. The prevalence and diversity of *CD274* CN changes in this study can serve as a basis for future clinical studies when further exploring *CD274* CN changes.

Both gains and losses of *CD274* were correlated with PD-L1 IHC status. This stands in contrast to genes like *ROS1*, where CN changes and *ROS1* protein expression detected via IHC are not highly correlated.²⁷ Instead, *CD274* CN gains are more similar to *ERBB2* (HER2) CN gains and HER2 protein expression in that they are correlated with each other.²⁷ Interestingly, HNSCC *CD274* CN gains had the highest correlation with PD-L1 IHC positivity. Furthermore, the highest levels of CN gains were in tumor types with SCC morphology, suggesting that *CD274* CN gains could be a particularly useful biomarker for tumors with this morphology. In our exploratory analysis of HNSCC and cervical SCC, we saw a positive correlation of HPV infection with *CD274* CN gain in these two tumor types. This suggests that the HPV infection likely caused the higher prevalence of *CD274* CN gains in HNSCC and cervical SCC, though the exact mechanism for this remains elusive and warrants further investigation.

Lastly, when we analyzed the correlation of TMB-H with *CD274* CN gains, we found that in almost all tumor types, there was no significant correlation between *CD274* CN gains and TMB-H which is consistent with the findings by Yarchoan *et al*²⁸ that PD-L1 expression and TMB are independent biomarkers in most tumor types. On the other hand, we saw significant correlation between *CD274* CN gains and MSI-H in the tumor types where MSI is most clinically relevant, but not in most other tumor types. Importantly, subsets of patients were negative for PD-L1 IHC but had *CD274* CN gain (also positive for PD-L1 IHC and had *CD274* loss). Also, we saw high specificity and positive predictive value of *CD274* CN positivity (with most CN cut-offs) with PD-L1 IHC positivity suggesting that *CD274* CN positivity is selecting patients who are likely to respond to ICPI. On the other hand, we observed relatively low sensitivity and negative predictive value of *CD274* CN positivity (at almost all CN cut-offs), meaning

that *CD274* CN positivity is only selecting a subset of the PD-L1 IHC positive tumors. These results in whole suggest that *CD274* CN changes could be an independent positive or negative predictive biomarker for ICPI response.

CONCLUSION

CD274 CN changes and PD-L1 expression were highly correlated in multiple tumor types. CGP-based *CD274* CN losses/gains obtained during routine clinical care could identify subsets of patients that are discordant with other known ICPI biomarkers, supporting further development of *CD274* CN losses/gains as a ICPI biomarker. These prevalence data on *CD274* CN changes across a large cohort of different solid tumors can be used to design future clinical studies to assess whether *CD274* CN changes could be a potential biomarker for ICPI.

Contributors Conception/design: RSPH, KM, JSR. Provision of study material or patients: RSPH, KM, MM, DCP, DAM, MH, BD, GF, LAA, JSR. Collection and/or assembly of data: RSPH, KM. Data analysis and interpretation: All authors. Manuscript writing: All authors. Final approval of manuscript: all authors.

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ORCID iD

Richard S.P. Huang <http://orcid.org/0000-0001-8395-5168>

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