Am J Cancer Res 2024;14(1):368-377
www.ajcr.us /ISSN:2156-6976/ajcr0154812

Brief Communication

LAG-3 transcriptomic expression correlates linearly with other checkpoints, but not with clinical outcomes

Jacob J Adashek[1*], Shumei Kato[2*], Daisuke Nishizaki[2], Sarabjot Pabla[3], Mary K Nesline[3], Rebecca A Previs[3], Jeffrey M Conroy[3], Paul DePietro[3], Razelle Kurzrock[4,5,6]

[1]Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins Hospital, Baltimore, MD 21287, USA; [2]Center for Personalized Cancer Therapy and Division of Hematology and Oncology, Department of Medicine, UC San Diego Moores Cancer Center, La Jolla, CA 92093, USA; [3]Labcorp Oncology, Durham, NC 27560, USA; [4]WIN Consortium, Paris 94550, France; [5]MCW Cancer Center, Milwaukee, WI 53226, USA; [6]University of Nebraska, Omaha, NE 68198, USA. [*]Equal contributors.

Received December 6, 2023; Accepted January 11, 2024; Epub January 15, 2024; Published January 30, 2024

Abstract: Immune checkpoint inhibitors have revolutionized the treatment landscape for patients with cancer. Multiomics, including next-generation DNA and RNA sequencing, have enabled the identification of exploitable targets and the evaluation of immune mediator expression. There is one FDA-approved LAG-3 inhibitor and multiple in clinical trials for numerous cancers. We analyzed LAG-3 transcriptomic expression among 514 patients with diverse cancers, including 489 patients with clinical annotation for their advanced malignancies. Transcriptomic LAG-3 expression was highly variable between histologies/cancer types and within the same histology/cancer type. LAG-3 RNA levels correlated linearly, albeit weakly, with high RNA levels of other checkpoints, including PD-L1 (Pearson's R^2 = 0.21 ($P < 0.001$)), PD-1 (R^2 = 0.24 ($P < 0.001$)) and CTLA-4 (R^2 = 0.19 ($P < 0.001$)); when examined for Spearman correlation, significance did not change. LAG-3 expression (dichotomized at ≥ 75th (high) versus < 75th (moderate/low) RNA percentile level) was not a prognostic factor for overall survival (OS) in 272 immunotherapy-naïve patients with advanced/metastatic disease (Kaplan Meier analysis; P = 0.54). High LAG-3 levels correlated with longer OS after anti-PD-1/PD-L1-based checkpoint blockade (univariate (P = 0.003), but not multivariate analysis (hazard ratio, 95% confidence interval = 0.80 (0.46-1.40) (P = 0.44))); correlation with longer progression-free survival showed a weak univariate trend (P = 0.13). Taken together, these results suggest that high LAG-3 levels in and of themselves do not predict resistance to anti-PD-1/PD-L1 checkpoint blockade. Even so, since LAG-3 is often co-expressed with PD-1, PD-L1 and/or CTLA-4, selecting patients for combinations of checkpoint blockade based on immunomic co-expression patterns is a strategy that merits exploration.

Keywords: LAG-3, cancer, biomarker, checkpoints, immunotherapy

Introduction

The immune checkpoint CD223 or lymphocyte activation gene 3 (LAG-3) is often present on CD4+ and CD8+ T cells, as well as natural killer cells, regulatory T cells (Tregs), and plasmacytoid dendritic cells [1]. LAG-3 functions as a co-receptor dampening the immune system; when aberrant, LAG-3 is implicated in autoimmune conditions, immune response, and carcinogenesis [2].

A vital component of the immunoglobulin superfamily, LAG-3 is located on human chromosome 12. LAG-3 is a type I transmembrane protein containing 498 amino acids, consisting of an extracellular region, a transmembrane region, and a cytoplasmic region [3]. LAG-3 plays a critical role in the adaptive immune response and is expressed on the surface of regulatory T cells (Tregs) and effector T cells, which regulate the interaction of T lymphocytes and antigen-presenting cells (APCs) [2]. Like CTLA-4 and PD-L1, LAG-3 is induced on CD8+ and CD4+ T cells under persistent antigenic stimulus rather than being expressed on naïve T cells [4]. The pro-inflammatory state that malignancy can create causes maintained exposure to antigens, leading to high levels of continuous expression of LAG-3 and additional inhibitory co-molecules on CD4+ and CD8+ T cells, which become exhausted and results in

https://doi.org/10.62347/MVDH5025

decreased responses, tumor killing, and upregulation of Treg immune dampening function [5]. Blocking LAG-3 can permit T cells to regain cytotoxic ability and reduce the immunosuppressive effects of Tregs, thereby enriching the killing capacity on tumors [1, 6].

LAG-3 is reported to have four ligands within the tumor microenvironment: (i) major histocompatibility complex II (MHC II); (ii) galactose lectin-3; (iii) fibrinogen-like protein 1 (FGL1); and (iv) hepatic sinusoid endothelial cell lectin [7]. The primary ligand of LAG-3 is MHC II, and it is also a common ligand for CD4 [8]. Interestingly, the binding affinity for LAG-3 and MHC II is 100 times higher than for MHC II and CD4; thus, LAG-3 and CD4 may bind competitively with MHC II and negatively regulate CD4 function [9, 10].

In general, inhibitory checkpoints such as CTLA-4, LAG-3, PD-1 and PD-L1 serve to protect the body from self-inflicted damage from the immune system. A mechanism that cancer can invoke to further growth and progression is by exploiting these same checkpoints to evade the immune system. Multiple agents are approved to target these checkpoints (CTLA-4, LAG-3, PD-1, and PD-L1) and clinically meaningful for a multitude of different cancer histologies [11-13].

Regardless of tumor histology, LAG-3 expression has been reported to be differentially expressed when aberrations in specific genes are found: *EZH2*, *CDKN2A*, and *MPL* [14]. Still, the majority of trials using anti-LAG-3 agents have not met primary endpoints [1]. LAG-3 protein and RNA expression has been correlated to cytokine-producing (interferon γ and interleukin-17A) and activated and T cell subsets and also positively associated with disease activity [15]. However, relatlimab, an anti-LAG-3 monoclonal antibody, in combination with nivolumab, an anti-PD-1 monoclonal antibody, was approved by the Food and Drug Administration (FDA) in March 2022 for patients with unresectable or metastatic melanoma based on the improvement in progression-free survival (PFS) 10.1 vs. 4.6 months (hazard ratio [HR] 0.75; 95% confidence interval [CI] 0.62-0.92; P = 0.006) [11]. Interestingly, analysis of biomarker-enriched population in the phase 1/2a study of relatlimab in combination with nivolumab showed higher responses correlating with LAG-3 expression (≥ 1%), irrespective of PD-L1 expression [16].

In this study, we ask if LAG-3 expression correlated to survival outcomes and/or with expression of other immunomodulatory effectors. This study specifically analyzed the impact of LAG-3 transcriptomic expression on outcome in the pan-cancer setting, specifically amongst 272 patients who never received immune checkpoint inhibitors (ICIs) and amongst 217 patients who received ICIs, as well as patterns of expression of LAG-3 and other clinically relevant checkpoints.

Materials and methods

The LAG-3 (CD223) RNA expression levels were analyzed from tissue derived from 514 locally advanced or metastatic tumor samples from the University of California San Diego (UCSD) clinic. Analysis was performed at a Clinical Laboratory Improvement Amendments (CLIA)-licensed and College of American Pathologist (CAP)-accredited clinical laboratory--OmniSeq (https://www.omniseq.com/). The study was conducted under the Study of Personalized Cancer Therapy to Determine Response and Toxicity, UCSD_PREDICT, NCT02478931 protocol, which obtained patient consent for any investigational interventions and followed the UCSD Institutional Review Board guidelines. The database has been previously described [17-20].

Samples underwent RNA sequencing at OmniSeq laboratory and were provided in formalin-fixed, paraffin-embedded (FFPE) after specimen collection. RNA extraction from FFPE was conducted by truXTRAC FFPE extraction kit (Covaris, Inc., Woburn, MA) and the manufacturer's instructions were followed. After purification, the RNA was dissolved in 50 μL water and the yield was measured via Quant-iT RNA HS assay (Thermo Fisher Scientific, Waltham, MA). A pre-defined titer of 10 ng of RNA was acceptable for preparation of the library. RNA expression absolute read count was estimated via Torrent Suite's plugin immuneResponseRNA (v5.2.0.0) 34. Background subtraction, percentile ranking, and normalization using custom scripts was performed [21]. Using an internal housekeeping gene profile dataset, transcript abundance was normalized and ranked (0-100 percentile levels) in reference to a data-

set of 735 tumors and across 35 tumor histologies reference dataset. Expression profiles were stratified by abundance of transcripts into "moderate/low" (0-74), and "high" (75-100) percentile LAG-3 RNA level.

Analysis of tumor mutational burden (TMB) was obtained via genomic DNA from FFPE tumors with > 30% tumor nuclei via truXTRAC FFPE extraction kit (Covaris) with 10 ng DNA input for library preparation. DNA Libraries were prepared with Ion AmpliSeq targeted sequencing chemistry employing the Comprehensive Cancer Panel, followed by enrichment and template preparation utilizing the Ion Chef system, and sequencing on the Ion S5XL 540 chip (Thermo Fisher Scientific). TMB was reported as mutations/megabase after removal of synonymous variants, germline variants, indels and single nucleotide variants with < 5% variant allele fraction.

Outcome endpoints and statistical analyses

Survival analyses were performed for patients with survival information using the Kaplan-Meier method. For prognostic evaluation, overall survival (OS) was defined as the duration from the date of metastatic or locally advanced disease to the date of the last follow-up. To assess the effect of LAG-3 on immunotherapy outcomes (predictive information), Kaplan-Meier analysis was limited to patients received immunotherapy as part of standard of care treatments. OS was defined as the duration from the initial date of immunotherapy to the date of last follow-up, and PFS was defined as the duration from the initial date of immunotherapy to the date of the earliest of disease progression (clinical or radiological) or death from any cause. Patients still alive (for OS) or progression free (for PFS) were censored at the point of last contact or date of data cut off, whichever came first. The data cutoff date for the current analysis was June 24, 2022. All statistical analyses were performed using R 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). A p value of ≤ 0.05 was considered statistically significant.

Results

Patient characteristics

There were 514 patients including 489 with advanced metastatic disease and clinical annotation [17-20]. Their median age was 61 years (range 24-93 years); 40% were women. The most frequent tumor types were colorectal (N = 140), pancreatic (N = 55) and breast cancer (N = 49) (**Figure 1A**); 217 patients received ICI therapy, including 199 who received an anti-PD-1/PD-L1 agent without another ICI, 16 who received an anti-PD-1/PD-L1 agent with an anti-CTLA-4 and two who received only an anti-CTLA-4.

LAG-3 transcript expression varied between and within tumor types

To interrogate LAG-3 expression across diverse cancer types, we performed a comprehensive analysis of LAG-3 transcript expression across our 514 patients. The percentile of the LAG-3 expression based on RNA level in each tumor was ranked on a scale of 1 to 100 normalized to the reference population of 735 tumors spanning 35 histologies, and classified into moderate/low (0-74), and high (75-100) (see also Methods).

Across cancers (N = 514 tumors), 22.6% of patients expressed high LAG-3 transcript levels (≥ 75% rank); tumor types that most expressed high LAG-3 (with ≥ 30% of tumors showing high LAG-3 levels) included neuroendocrine tumors, uterine cancer, sarcoma, breast, and ovarian cancers (**Figure 1B**). Overall, 50% of melanomas showed high LAG-3 transcripts, but only six samples were available for analysis.

LAG-3 levels correlate with transcriptomic levels of other checkpoints

LAG-3 RNA levels correlated linearly with high levels of other checkpoints, including PD-L1 (Pearson's R^2 is 0.21 ($P < 0.001$)), PD-1 (R^2 is 0.24 ($P < 0.001$)) and CTLA-4 (R^2 is 0.19 ($P < 0.001$)) (**Figure 2**). However, some of these correlations were weak. LAG-3 did not correlate significantly with TMB or with IL-17A levels (**Figure 2**). When examined for Spearman correlation, significance did not change (**Figure 2**).

LAG-3 levels were not a prognostic factor for survival in patients who never received immunotherapy

Figure 3 demonstrates that high LAG-3 RNA expression was associated with better prognosis (longer survival from diagnosis or metastatic/advanced disease; P = 0.041) in the full

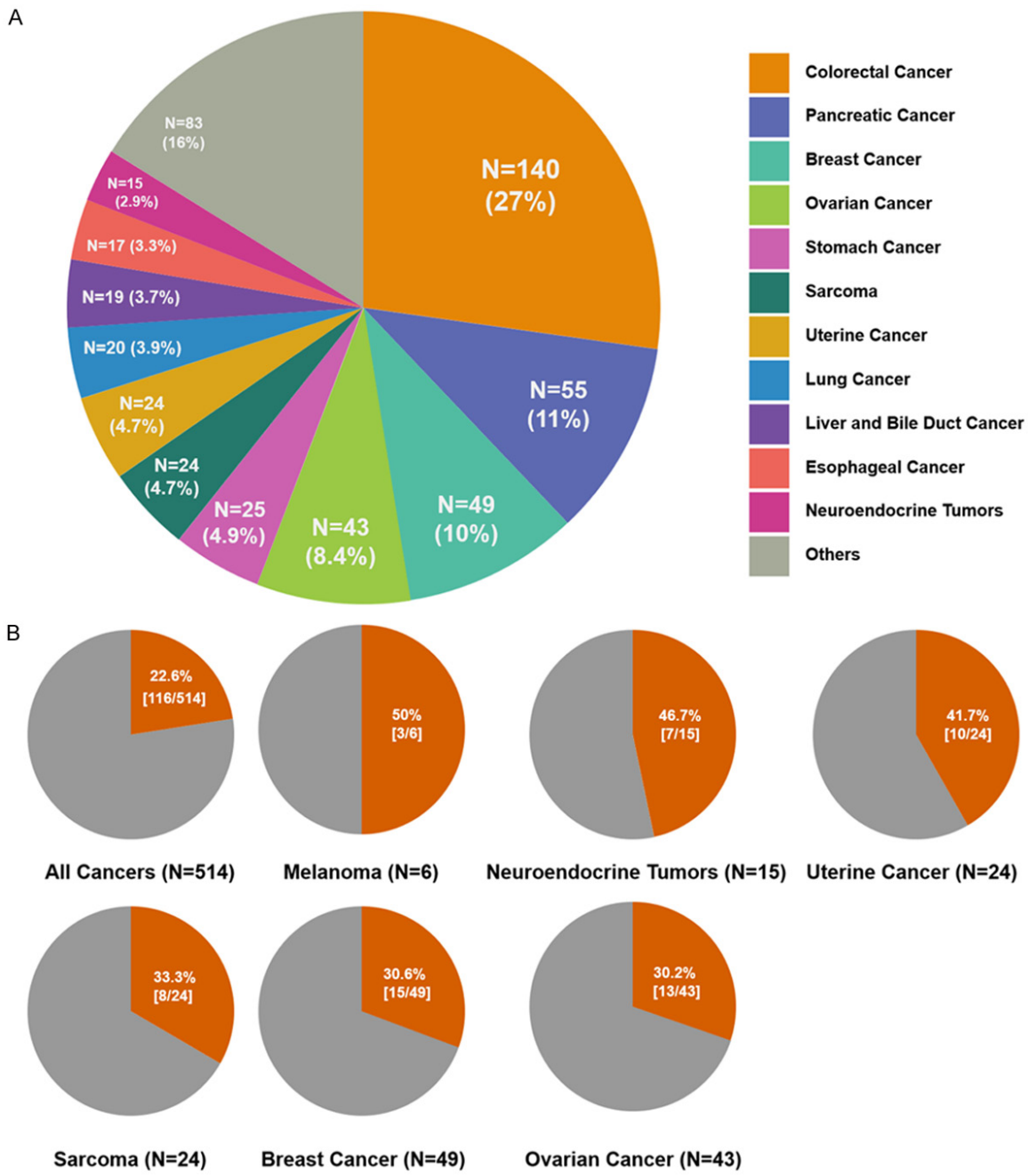

Figure 1. A. Types of cancers analyzed (N = 514). Tumor types with ≥ 15 samples are shown. B. Percent of patient with high LAG-3 (≥ 75 percentile RNA level) in various tumor types. Tumor types with ≥ 15 samples, and with ≥ 30% of tumors showing high LAG-3 are depicted. Because of FDA approval of relatlimab (anti-LAG-3) in melanoma, melanoma is also shown, even though less samples were available.

489 patients. There was no significant difference in survival outcomes when the 217 patients who received immunotherapy were removed (leaving 272 patients who never received an ICI) (P = 0.54). Immunotherapy itself was not a prognostic factor in the full 489 patients (P = 0.4).

High LAG-3 levels associated with longer survival after immune checkpoint inhibitor treatment in univariable but not in multivariable analysis

High (≥ 75th percentile RNA rank) versus moderate/low LAG-3 levels were significantly associ-

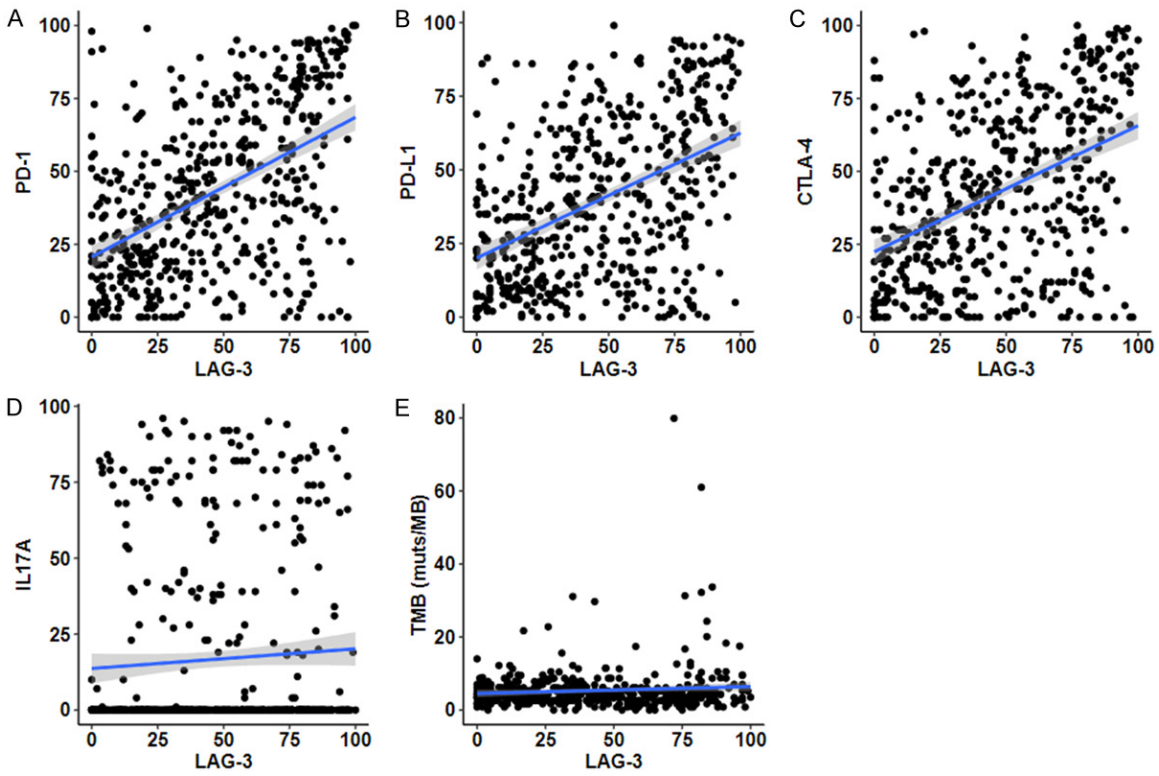

Figure 2. Scatter plots of LAG-3 versus PD-1, PD-L1, CTLA-4, IL17A, and TMB. Correlation between LAG-3 and other checkpoints' RNA percentile level as well as with TMB. A. LAG-3 vs. PD-1 percentile score. Pearson R^2 is 0.24 ($P < 0.001$). Spearman rho is 0.47 ($P < 0.001$) (not shown). B. LAG-3 vs. PD-L1 percentile score. Pearson R^2 is 0.21 ($P < 0.001$). Spearman rho is 0.44 ($P < 0.001$) (not shown). C. LAG-3 vs. CTLA-4 percentile score. Pearson R^2 is 0.19 ($P < 0.001$). Spearman rho is 0.43 ($P < 0.001$) (not shown). D. LAG-3 vs. IL-17A percentile score (used as a control). Pearson R^2 is 0.004 ($P = 0.16$). Spearman rho is 0.08 ($P = 0.08$) (not shown). E. LAG-3 percentile score vs. TMB score. Pearson's R^2 is 0.007 ($P = 0.07$). Spearman's rho is -0.04 ($P = 0.41$) (not shown). Abbreviations: MB, megabase; muts, mutations; TMB, tumor mutational burden.

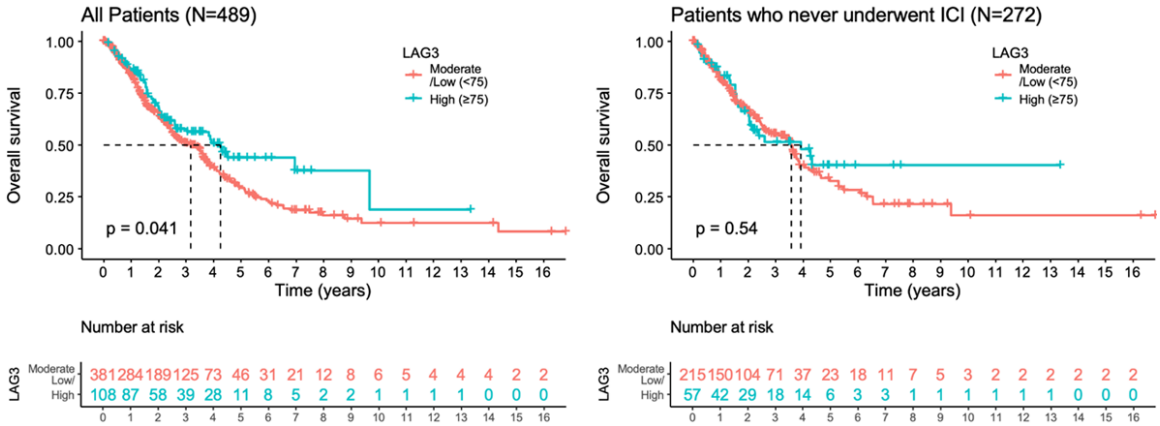

Figure 3. Prognostic impact of high LAG-3 RNA levels. High LAG-3 was defined as ≥ 75 percentile RNA level (see Methods). Overall survival was calculated from time of metastatic/locally advanced disease to death. The figures show that high LAG-3 RNA expression was associated with better prognosis (longer survival) in the full 489 patients, but not when the 217 patients who received ICI were removed (leaving 272 patients who never received an ICI).

ated with longer OS (from the start of ICI treatment) in the 217 patients treated with an ICI (median OS was 1.94 years in high LAG-3 patients and 1.24 years in moderate/low LAG-3

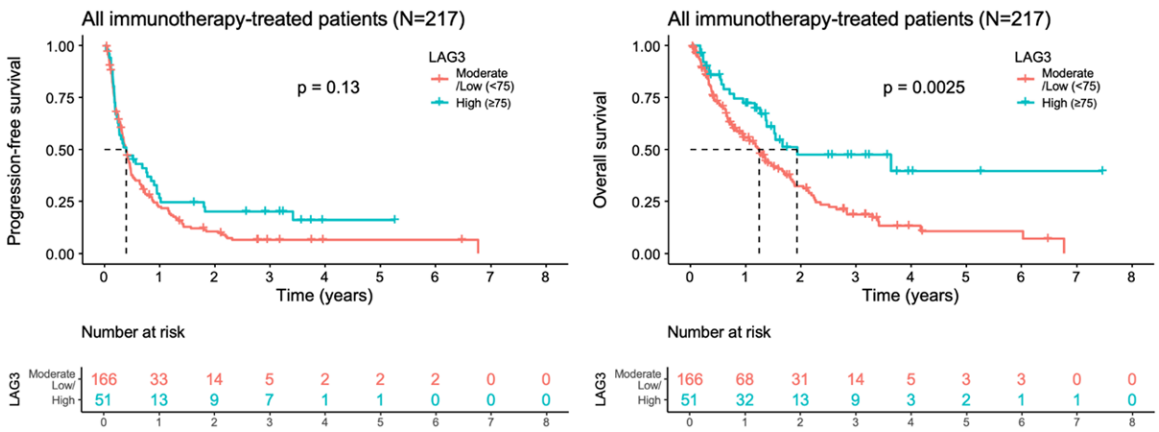

Figure 4. Outcomes after ICI in patients with high LAG-3 versus moderate/low LAG-3. High LAG-3 was defined as ≥ 75 percentile level and moderate/low as < 75 percentile level. Progression-free survival (PFS) was defined as the duration from the date of immunotherapy initiation to the date of earliest of disease progression or death. OS was defined as the duration from the date of immunotherapy initiation to the date of death. Median PFS was 0.39 years in high LAG-3 patients and 0.39 years in moderate/low LAG-3 patients, respectively (P = 0.13, log-rank test). Median OS was 1.94 years in high LAG-3 patients and 1.24 years in moderate/low LAG-3 patients, respectively (P = 0.0025, log-rank test).

patients, respectively (P = 0.0025, log-rank test)) (**Figure 4**). Results were not changed when the two patients treated with an anti-CTLA-4 by itself were excluded. There was a trend toward correlation with longer PFS, but it did not reach statistical significance (P = 0.13). The correlation between high LAG-3 RNA levels and longer OS after ICI treatment was not retained as an independent factor in Cox regression analysis (P = 0.44) (**Table 1**).

Discussion

We observed that the greatest percentage of patients with high LAG-3 RNA expression occurred in melanoma, uterine, colorectal, pancreatic, breast, ovarian, stomach, sarcoma, lung, liver and bile duct, esophageal, and neuroendocrine tumors (**Figure 1**); in each of these tumor types, ≥ 30% of patients had high LAG-3 expression, defined as ≥ 75th percentile rank, which could have implications for future trial design for specific cancers. However, even in these cancers, most cancers did not express high LAG-3. Overall, high LAG-3 levels were observed in ~23% of patients across malignancies. These findings reflect the variability of LAG-3 transcriptomic expression between and within cancer types, and the need to examine checkpoint expression in each tumor if information about expression level is to be utilized.

In the current analysis, high LAG-3 transcripts were linearly correlated with high transcripts of other checkpoints such as PD-1, PD-L1 and CTLA-4 (**Figure 2**). These results are consistent with our prior study indicating that LAG-3 and the other checkpoints named above correlate independently when the levels are dichotomized as high versus moderate/low [17]. These associations may point to possible benefit of combining LAG-3 with anti-PD-1/PD-L1 agents or anti-CTLA-4 agents, depending on which checkpoints are co-expressed in individual tumors. Indeed, the immune inhibitory molecules LAG-3 and PD-1 have been reported to synergistically regulate T cell function in order to promote tumoral immune escape [22]. Moreover, the anti-LAG-3 relatlimab, plus the anti-PD-1 nivolumab, showed superior outcomes to nivolumab alone in melanoma, leading to FDA approval [11]. Further, compensatory upregulation of PD-1, LAG-3, and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer [23]. Examining individual tumors for immunomic expression and choosing specific checkpoint blockade for patients based on their tumor immune portraits may therefore warrant exploration.

Our prior studies also demonstrated independent correlation between high (≥ 10 mutations/mb) versus low TMB (an important biomarker for immunotherapy response) [24] and high (≥ 75th percentile RNA rank) versus low/moderate LAG-3 [17]; in our current study, this correlation is not significant when both variables examined are interrogated in a linear (rather than dichoto-

Table 1. Univariable and multivariable Cox regression for overall survival among patients who received ICI (N = 217)

Variable	Condition	Univariable				Multivariable			
		Hazard Ratio	95% CI		*P*-value	Hazard Ratio	95% CI		*P*-value
			Lower	Upper			Lower	Upper	
Age	< 61 years	-							
	≥ 61 years	1.05	0.75	1.48	0.77				
Sex	Female	-							
	Male	1.09	0.78	1.54	0.61				
LAG-3	Low/Moderate	-				-			
	High	0.50	0.32	0.79	0.003	0.80	0.46	1.40	0.44
PD-L1	Low/Moderate	-				-			
	High	0.58	0.33	1.01	0.054	0.81	0.39	1.67	0.57
PD-L2	Low/Moderate	-				-			
	High	0.59	0.38	0.92	0.02	0.78	0.43	1.41	0.40
CTLA-4	Low/Moderate	-				-			
	High	0.44	0.27	0.74	0.002	0.56	0.28	1.12	0.10
TMB	< 10 muts/MB	-				-			
	≥ 10 muts/MB	0.59	0.32	1.07	0.08	0.55	0.3	1.03	0.06
MSI	Stable					-			
	Unstable	0.35	0.11	1.09	0.07	0.38**	0.12	1.23	0.11
Colorectal cancer	No	-				-			
	Yes	1.70	1.16	2.49	0.007	1.79	1.17	2.74	0.01 Worse outcome
Breast cancer	No	-							
	Yes	0.85	0.45	1.63	0.63				
Ovarian cancer	No	-							
	Yes	1.04	0.59	1.85	0.88				
Pancreatic cancer	No	-				-			
	Yes	1.70	0.92	3.16	0.09	1.70	0.82	3.54	0.15
Uterine cancer	No	-							
	Yes	0.72	0.32	1.62	0.42				
Sarcoma	No	-				-			
	Yes	0.49	0.20	1.22	0.13	0.53	0.2	1.40	0.20
Neuroendocrine tumor	No	-							
	Yes	0.81	0.26	2.55	0.72				
Lung cancer	No	-							
	Yes	0.90	0.47	1.74	0.76				

Footnotes: Overall survival was defined as duration from the date of ICI initiation to the date of death. Variables with P < 0.2 from univariate analyses were included in the multivariate analysis. **MSI and TMB have a multicollinearity and we performed two sets of multivariable models: Model 1 included TMB and other seven variables (LAG-3, PD-L1, CTLA-4, colorectal cancer, pancreatic cancer, and sarcoma) and Model 2 included MSI and the other seven variables. The estimate and corresponding CI and *p*-value of MSI were derived from Model 2, while others were derived from Model 1. Abbreviations: CI, confidence interval; MB, megabase; MSI, microsatellite instability; muts, mutations; TMB, tumor mutation burden.

mized) fashion, suggesting perhaps a threshold TMB effect on such an association.

LAG-3 expression and prognosis has been examined in various tumors with conflicting results. As reviewed by Shi and colleagues [25], high levels of LAG-3 were a harbinger of a poor prognosis in some cancers such as non-small cell lung cancer and hepatocellular carcinoma; however, in gastric cancer, high LAG-3 predicted a superior prognosis [26-28]. In another study, high LAG-3 correlated with better prog-

nosis (longer survival) across cancers [29]. In our study, high LAG-3 levels correlated with longer survival (from diagnosis of advanced/metastatic disease) when all 489 patients were evaluated, but not when only the 272 patients who never received ICI were assessed (**Figure 3**). Since the 217 patients who received ICI demonstrated longer survival (from immunotherapy treatment start date) when LAG-3 levels were high versus moderate/low (**Figure 4**), it is plausible that high LAG-3 levels are not a prognostic factor, consistent with our results, but that ICI treatment may confound the prognostic implications, and this may also explain why some prior studies showed an association between high LAG-3 levels and better prognosis.

Although the 217 patients who received ICIs demonstrated significantly longer survival when LAG-3 was high (P = 0.0025), this correlation was not retained in multivariate analysis, suggesting that extraneous elements (perhaps other checkpoints) with which LAG-3 co-segregates are more critical. However, this result is of interest; we previously hypothesized that high LAG-3 levels might mediate resistance to anti-PD-1/PD-L1 therapies [17]. If that were the case, we would expect high LAG-3 levels to associate with poorer outcomes after anti-PD-1/PD-L1 treatment, but the opposite was found, at least in univariate analysis; and in multivariate analysis, LAG-3 levels had a null effect on outcome after anti-PD-1/PD-L1 agents. Therefore, it appears that high levels of LAG-3 transcripts are not associated with resistance to checkpoint blockade with PD-1/PD-L1 antibodies.

Our study has several limitations. First, the sample size for individual histologies was small, allowing analysis across cancers, but not robust analysis of individual cancer types. Analysis of individual tumor types will be important for future studies. Second, while RNA interrogation may give important information about expressed versus silenced transcript expression [30, 31], there is not always a clean correlation between RNA and protein expression, and our study did not examine protein levels. Finally, the cell type expressing LAG-3 may be important and should be addressed in future research.

In summary, there is one FDA-approved LAG-3 inhibitor (relatlimab) and multiple clinical trials of LAG-3 inhibitors for various cancer types. Many ongoing studies and the current FDA approval are for a LAG-3 inhibitor in combination with another checkpoint (such as the anti-PD-1 inhibitor nivolumab) [11]. Our study shows that LAG-3 expression varies between and within tumor types. Importantly, there is a linear relationship between LAG-3 RNA expression and that of other critical checkpoints such as PD-1, PD-L1 and CTLA-4. Higher LAG-3 levels by themselves were not a prognostic factor for survival in patients who were immunotherapy naïve. In patients treated with anti-PD-1/PD-L1-based regimens, high LAG-3 transcripts predicted longer survival in univariate but not in multivariate analysis, possibly because of confounding by high co-expression of other checkpoints such as PD-1 and PD-L1. Future studies of combination checkpoint blockade may want to select high co-expressors for treatment in order to determine if expression levels correlate with enhanced responsiveness when specific checkpoints are co-targeted.

Acknowledgements

Razelle Kurzrock is funded in part by 5U01CA180888-08 and 5UG1CA233198-05. Shumei Kato is funded in part by the National Cancer Institute grant P30 CA023100.

Disclosure of conflict of interest

Jacob J Adashek serves on the advisory board of CureMatch Inc. and serves as a consultant for datma. Shumei Kato serves as a consultant for Medpace, Foundation Medicine, NeoGenomics and CureMatch. He receives speaker's fee from Roche and Bayer, and advisory board for Pfizer. He has research funding from ACT Genomics, Sysmex, Konica Minolta, OmniSeq and Personalis. Sarabjot Pabla, Mary K Nesline, Jeffrey M Conroy, Paul DePietro are/were employees of Labcorp Oncology. Rebecca A Previs is/was an employee of Labcorp and declares the following relationships: stock ownership from Labcorp. She has served on advisory boards for Myraid Genetics and Natera. Razelle Kurzrock has received research funding from Biological Dynamics, Boehringer Ingelheim, Debiopharm, Foundation Medicine, Genentech, Grifols, Guardant, Incyte, Konica Minolta, Medimmune, Merck Serono, Omniseq, Pfizer, Sequenom, Takeda, and TopAlliance; as well as consultant and/or speaker fees and/or advisory board for Actuate Therapeutics,

AstraZeneca, Bicara Therapeutics, Biological Dynamics, Caris, Daiichi Sankyo, Inc., Datar Cancer Genetics, EISAI, EOM Pharmaceuticals, Iylon, LabCorp, Merck, NeoGenomics, Neomed, Pfizer, Precirix, Prosperdtx, Roche, TD2/Volastra, Turning Point Therapeutics, X-Biotech; has an equity interest in CureMatch Inc., CureMetrix, and IDbyDNA; serves on the Board of CureMatch and CureMetrix, and is a co-founder of CureMatch.

Address correspondence to: Dr. Jacob J Adashek, Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins Hospital, Baltimore, MD 21287, USA. E-mail: jadashek@westernu.edu

References

[1] Ruffo E, Wu RC, Bruno TC, Workman CJ and Vignali DAA. Lymphocyte-activation gene 3 (LAG3): the next immune checkpoint receptor. Semin Immunol 2019; 42: 101305.

[2] Maruhashi T, Sugiura D, Okazaki IM and Okazaki T. LAG-3: from molecular functions to clinical applications. J Immunother Cancer 2020; 8: e001014.

[3] Triebel F. LAG-3: a regulator of T-cell and DC responses and its use in therapeutic vaccination. Trends Immunol 2003; 24: 619-622.

[4] Qin S, Xu L, Yi M, Yu S, Wu K and Luo S. Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4. Mol Cancer 2019; 18: 155.

[5] Anderson AC, Joller N and Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. Immunity 2016; 44: 989-1004.

[6] Puhr HC and Ilhan-Mutlu A. New emerging targets in cancer immunotherapy: the role of LAG3. ESMO Open 2019; 4: e000482.

[7] Huo JL, Wang YT, Fu WJ, Lu N and Liu ZS. The promising immune checkpoint LAG-3 in cancer immunotherapy: from basic research to clinical application. Front Immunol 2022; 13: 956090.

[8] Baixeras E, Huard B, Miossec C, Jitsukawa S, Martin M, Hercend T, Auffray C, Triebel F and Piatier-Tonneau D. Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens. J Exp Med 1992; 176: 327-337.

[9] Huard B, Prigent P, Tournier M, Bruniquel D and Triebel F. CD4/major histocompatibility complex class II interaction analyzed with CD4- and lymphocyte activation gene-3 (LAG-3)-Ig fusion proteins. Eur J Immunol 1995; 25: 2718-2721.

[10] Weber S and Karjalainen K. Mouse CD4 binds MHC class II with extremely low affinity. Int Immunol 1993; 5: 695-698.

[11] Tawbi HA, Schadendorf D, Lipson EJ, Ascierto PA, Matamala L, Castillo Gutierrez E, Rutkowski P, Gogas HJ, Lao CD, De Menezes JJ, Dalle S, Arance A, Grob JJ, Srivastava S, Abaskharoun M, Hamilton M, Keidel S, Simonsen KL, Sobiesk AM, Li B, Hodi FS and Long GV; RELATIVITY-047 Investigators. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. N Engl J Med 2022; 386: 24-34.

[12] Chang E, Pelosof L, Lemery S, Gong Y, Goldberg KB, Farrell AT, Keegan P, Veeraraghavan J, Wei G, Blumenthal GM, Amiri-Kordestani L, Singh H, Fashoyin-Aje L, Gormley N, Kluetz PG, Pazdur R, Beaver JA and Theoret MR. Systematic review of PD-1/PD-L1 inhibitors in oncology: from personalized medicine to public health. Oncologist 2021; 26: e1786-e1799.

[13] Saad P and Kasi A. Ipilimumab. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022.

[14] Szeto CW, Kurzrock R, Kato S, Goloubev A, Veerapaneni S, Preble A, Reddy SK and Adashek JJ. Association of differential expression of immunoregulatory molecules and presence of targetable mutations may inform rational design of clinical trials. ESMO Open 2022; 7: 100396.

[15] Slevin SM, Garner LC, Lahiff C, Tan M, Wang LM, Ferry H, Greenaway B, Lynch K, Geremia A, Hughes S, Leavens K, Krull D, Marks DJB, Nevin K, Page K, Srinivasan N, Tarzi R, Klenerman P, Travis S, Arancibia-Carcamo CV and Keshav S. Lymphocyte activation gene (LAG)-3 is associated with mucosal inflammation and disease activity in ulcerative colitis. J Crohns Colitis 2020; 14: 1446-1461.

[16] Ascierto PA, Bono P, Bhatia S, Melero I, Nyakas MS, Svane IM, Larkin J, Gomez-Roca C, Schadendorf D, Dummer R, Marabelle A, Hoeller C, Maurer M, Harbison CT, Mitra P, Suryawanshi S, Thudium K and Muñoz Couselo E. Efficacy of BMS-986016, a monoclonal antibody that targets lymphocyte activation gene-3 (LAG-3), in combination with nivolumab in Pts with melanoma who progressed during prior anti-PD-1/PD-L1 therapy (mel prior IO) in all-comer and biomarker-enriched populations. Ann Oncol 2017; 28: v611-v612.

[17] Adashek JJ, Kato S, Nishizaki D, Miyashita H, De P, Lee S, Pabla S, Nesline M, Conroy JM, DePietro P, Lippman S and Kurzrock R. LAG-3 transcriptomic expression patterns across malignancies: implications for precision immunotherapeutics. Cancer Med 2023; 12: 13155-13166.

[18] Fujiwara Y, Kato S, Nesline MK, Conroy JM, DePietro P, Pabla S and Kurzrock R. Indoleamine 2,3-dioxygenase (IDO) inhibitors and cancer immunotherapy. Cancer Treat Rev 2022; 110: 102461.
[19] Miyashita H, Kurzrock R, Bevins NJ, Thangathurai K, Lee S, Pabla S, Nesline M, Glenn ST, Conroy JM, DePietro P, Rubin E, Sicklick JK and Kato S. T-cell priming transcriptomic markers: implications of immunome heterogeneity for precision immunotherapy. NPJ Genom Med 2023; 8: 19.
[20] Jou J, Kato S, Miyashita H, Thangathurai K, Pabla S, DePietro P, Nesline MK, Conroy JM, Rubin E, Eskander RN and Kurzrock R. Cancer-immunity marker RNA expression levels across gynecologic cancers: implications for immunotherapy. Mol Cancer Ther 2023; 22: 1352-1362.
[21] Conroy JM, Pabla S, Glenn ST, Burgher B, Nesline M, Papanicolau-Sengos A, Andreas J, Giamo V, Lenzo FL, Hyland FCL, Omilian A, Bshara W, Qin M, He J, Puzanov I, Ernstoff MS, Gardner M, Galluzzi L and Morrison C. Analytical validation of a next-generation sequencing assay to monitor immune responses in solid tumors. J Mol Diagn 2018; 20: 95-109.
[22] Woo SR, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, Bettini ML, Gravano DM, Vogel P, Liu CL, Tangsombatvisit S, Grosso JF, Netto G, Smeltzer MP, Chaux A, Utz PJ, Workman CJ, Pardoll DM, Korman AJ, Drake CG and Vignali DA. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res 2012; 72: 917-927.
[23] Huang RY, Francois A, McGray AR, Miliotto A and Odunsi K. Compensatory upregulation of PD-1, LAG-3, and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer. Oncoimmunology 2016; 6: e1249561.
[24] Jardim DL, Goodman A, de Melo Gagliato D and Kurzrock R. The challenges of tumor mutational burden as an immunotherapy biomarker. Cancer Cell 2021; 39: 154-173.
[25] Shi AP, Tang XY, Xiong YL, Zheng KF, Liu YJ, Shi XG, Lv Y, Jiang T, Ma N and Zhao JB. Immune checkpoint LAG3 and its ligand FGL1 in cancer. Front Immunol 2022; 12: 785091.
[26] He Y, Yu H, Rozeboom L, Rivard CJ, Ellison K, Dziadziuszko R, Suda K, Ren S, Wu C, Hou L, Zhou C and Hirsch FR. LAG-3 protein expression in non-small cell lung cancer and its relationship with PD-1/PD-L1 and tumor-infiltrating lymphocytes. J Thorac Oncol 2017; 12: 814-823.
[27] Guo M, Qi F, Rao Q, Sun J, Du X, Qi Z, Yang B and Xia J. Serum LAG-3 predicts outcome and treatment response in hepatocellular carcinoma patients with transarterial chemoembolization. Front Immunol 2021; 12: 754961.
[28] Park Y, Seo AN, Koh J, Nam SK, Kwak Y, Ahn SH, Park DJ, Kim HH and Lee HS. Expression of the immune checkpoint receptors PD-1, LAG3, and TIM3 in the immune context of stage II and III gastric cancer by using single and chromogenic multiplex immunohistochemistry. Oncoimmunology 2021; 10: 1954761.
[29] Saleh RR, Peinado P, Fuentes-Antras J, Perez-Segura P, Pandiella A, Amir E and Ocana A. Prognostic value of lymphocyte-activation gene 3 (LAG3) in cancer: a meta-analysis. Front Oncol 2019; 9: 1040.
[30] Adashek JJ, Kato S, Parulkar R, Szeto CW, Sanborn JZ, Vaske CJ, Benz SC, Reddy SK and Kurzrock R. Transcriptomic silencing as a potential mechanism of treatment resistance. JCI Insight 2020; 5: e134824.
[31] Boichard A, Lippman SM and Kurzrock R. Therapeutic implications of cancer gene amplifications without mRNA overexpression: silence may not be golden. J Hematol Oncol 2021; 14: 201.