ABSTRACT 4582

MULTI-OMIC ANALYSIS OF THE PROGNOSTIC AND PREDICTIVE VALUE OF *LAG3* EXPRESSION IN UROTHELIAL CARCINOMA

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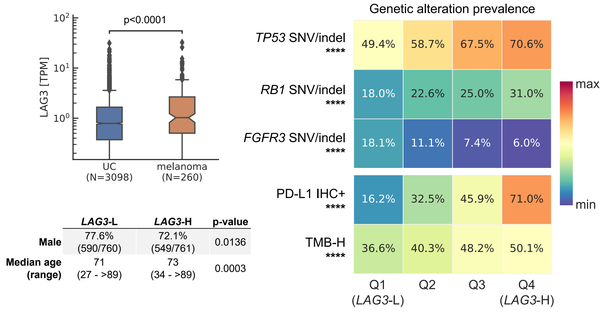
INTRODUCTION

- LAG3 is an immune checkpoint receptor protein expressed on immune cells, and LAG3 negatively regulates T-cell function and can promote tumor cell immune escape
- Co-blockade of PD1 and LAG3 enhanced anti-tumor responses in preclinical mouse models (ovarian cancer and colon adenocarcinoma)
- Nivolumab (anti-PD1) and Relatlimab (anti-LAG3) combination therapy has been approved to treat metastatic melanoma
- Despite its established prognostic significance in other malignancies, the role of *LAG3* as a prognostic or predictive biomarker in urothelial carcinoma (UC) remains inadequately studied

METHODS

- DNA (592-gene or whole exome) and RNA (whole transcriptome) sequencing were performed for 3343 patient tumors submitted to *Caris Life Sciences*
- Clinical and molecular data from patients enrolled in the *Oncology Research Information Exchange Network (ORIEN),* including tumor RNA-seq sequencing, were used as a validation cohort for analysis
- In this study, we investigate the correlation between LAG3 expression levels in tumor tissues of patients diagnosed with urothelial carcinoma and a range of clinicopathological parameters. We also assessed the impact on overall survival and response to standard therapies, aiming to elucidate potential mechanisms of resistance.
- PD-L1+ status (22c3, combined positive score ≥10) was determined by IHC and TMB-High (TMB-H) was defined as ≥10 mutations/Mb
- *LAG3* high (*LAG3*-H) and low (*LAG3*-L) cohorts were defined by the top and bottom quartiles of *LAG3* RNA transcripts per million (TPM), respectively
- Tumor microenvironment (TME) cell fractions were estimated by RNA deconvolution using quanTIseq
- Significance was tested using Mann-Whitney U and $\chi 2$ tests with multiple testing correction as appropriate
- Real-world overall survival (OS) was obtained from insurance claims data and calculated from start of Atezolumab, Avelumab, Nivolumab or Pembrolizumab to last contact.
- Pembrolizumab time on treatment (ToT) was calculated from start of Pembrolizumab to last of Pembrolizumab
- Hazard ratios (HR) and associated p-values were calculated using the Cox proportional hazards model
- Multivariate analysis (MVA) was performed using a Cox Proportional-Hazards model

RESULTS



	LAG3-L	LAG3-H	p-value
Male	77.6% (590/760)	72.1% (549/761)	0.0136
Median age (range)	71 (27 - >89)	73 (34 - >89)	0.0003

Figure 1. LAG3 expression in UC and association with increased TP53 mutations and PD-L1+ and TMB-H status

- (Top left) *LAG3* expression in UC vs metastatic melanoma patients treated with Nivolumab + Relatlimab combination therapy
- (Bottom left) Demographics of *LAG3*-H and *LAG3*-L cohorts
- (Right) Prevalence of genetic alterations vs *LAG3* expression quartile. Q-values for *LAG3*-H vs *LAG3*-L comparisons

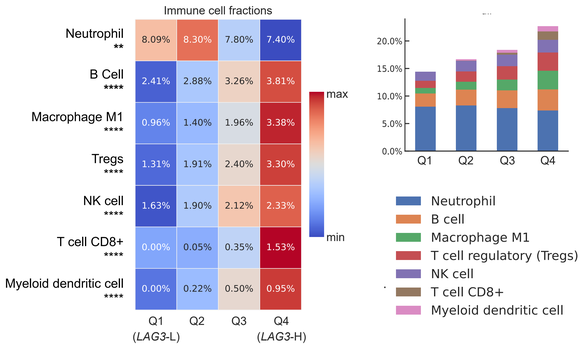


Figure 2. *LAG3*-H cohort had increased immune cell infiltration and increased Tregs

- (Left) Immune cell fractions vs *LAG3* expression quartile
- (Right) Total immune cell fractions by *LAG3* expression quartile. Stars represent q-values for *LAG3*-H vs *LAG3*-L comparisons

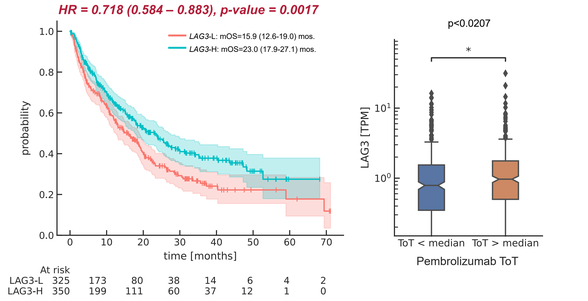


Figure 3. High *LAG3* expression associated with longer OS and Pembrolizumab ToT

- (Left) Post-ICI OS for *LAG3*-H vs *LAG3*-L cohorts
- (Right) *LAG3* expression in cohorts stratified by Pembrolizumab ToT

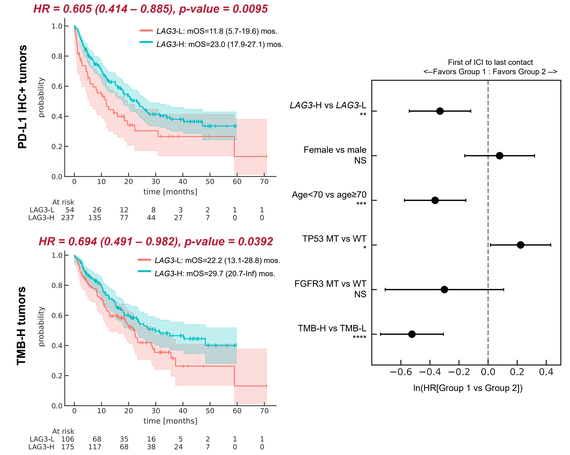


Figure 4. Longer OS for *LAG3*-H cohort persisted in PD-L1+ and TMB-H tumors

- Post-ICI OS for *LAG3*-H vs *LAG3*-L cohorts among PD-L1 IHC+ tumors (top left) and TMB-H tumors (bottom left)
- (Right) HRs and 95% CI from Multivariate analysis

VALIDATION COHORT

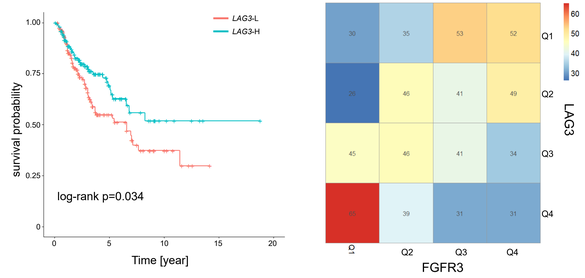


Figure 5. Validation cohort utilizing the ORIEN database

- (Left) OS for *LAG3*-H vs *LAG3*-L cohorts in ORIEN database
- (Right) *LAG3* expression vs prevalence of *FGFR3* mutations (spearman correlation coefficient = -0.205, p<0.0001)

CONCLUSIONS

- In UC, high *LAG3* expression is associated with improved survival and a hot tumor micro-environment
- Among patients with TMB-H, who received immune checkpoint inhibitors (ICI), *LAG3*-H had improved OS vs *LAG3*-L (HR=0.69, p=0.039).
- High *LAG3* is associated with increased *TP53* and *RB1* mutations, decreased *FGFR3* mutations, and increased PD-L1 IHC+ and TMB-H status
- UC patients may benefit from combination ICI because high *LAG3* expression is associated with increased immune cell infiltration, but also higher levels of inhibitory Tregs

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