Background: Despite recent advancements in the clinical use of immunotherapies, the role of exhausted T cells in the immunobiology of uveal melanoma is still unclear. Exhausted T lymphocytes in the tumor microenvironment exhibit activation of inhibitory receptors, decreased effector cytokine production, and cytolytic activity, resulting in failure of cancer cell elimination. Therefore, the goal of this study was to examine the predictive value of LAG3, CTLA-4, and T-cell markers (CD4, CD8, CD3, and FOXP3) expression in UM patients.

Methods: Prospective analysis of 54 UM tumor and blood specimens were taken for this study. Immunohistochemical analysis of exhausted T-cell targets (LAG3 and CTLA-4), and T-cell markers (CD3, CD4, CD8, and FOXP3) were performed on formalin-fixed paraffin-embedded specimens, and expression of exhausted T-cell targets were validated by western blotting. Transcriptional analysis was investigated by qRT-PCR on all the cases for LAG3 and CTLA-4 genes. We also checked the expression of CTLA-4, CCR8, and LAG3 at serum levels by sandwich-ELISA assay. Statistical analysis was performed to correlate tumor targets with clinicopathological parameters and patient outcomes.

Results: CD3, CD4, CD8, and FOXP3 expression was found in 41%, 35%, 50%, and 39% of the patients, respectively. TIL-positive UM appears to have more CD8-positive cells than other TIL markers. Furthermore, patients lacking nBAP1 who had these TILs exhibited aggressive behavior, which may be associated to shorter metastasis-free survival (MFS). LAG3 and CTLA-4 expression was found in both tumor cells and the lymphocytic environment of UM tissues. Higher LAG3 expression was found to be statistically significant with the presence of epithelioid cells (p=0.033), a high mitotic count (p=0.006), CD34 positive (p=0.001), and nBAP1 loss (p=0.007), respectively. While CTLA-4 expression was associated with ciliary body invasion (p=0.013) and CD34 positivity (p=0.031). Similarly, to protein expression, increased mRNA expression of LAG3 and CTLA-4 gene was found in high-risk UM. sLAG3 was considerably higher in metastatic UM (MUM) than in primary UM (PUM) (p<0.001). sCCR8 levels were significantly lower in MUM (p<0.05) than in PUM. Our data suggest that MFS rates were 60% and 57% in patients having LAG3 immunoexpression and BAP1 loss, respectively. MFS rates were 80% and 56%, respectively, for those who had mRNA expression of LAG3 and CTLA-4 with nBAP1 loss.

Conclusions: Our findings suggest LAG3 as a promising biomarker to specifically identify functionally exhausted T cells and has a prognostic significance in UM.

Ethics Approval: This study was approved by Institute’s Ethical Committee, AIIMS (Ref. No. IEC-424/RP-6/2016)

Consent: Written consent was obtained from all the patient’s guardian