Background The phase 2/3 RELATIVITY-047 clinical trial (NCT03470922) in patients with previously untreated metastatic or unresectable melanoma met its primary endpoint of improved progression-free survival with a combination of nivolumab plus relatlimab (NIVO+RELA) compared with NIVO monotherapy.\(^1\) To better understand the biology underlying combined programmed death-1 (PD-1) and lymphocyte activation gene 3 (LAG-3) inhibition, a series of exploratory biomarker analyses was performed. Analyses included longitudinal changes in immunomodulatory cytokines, free soluble LAG-3 (sLAG-3), and selected immune-cell markers within tumor biopsies collected at baseline.

Methods Peripheral blood serum samples collected prior to treatment, and prior to study drug during week 4 and week 8 of treatment, were evaluated for changes in inflammatory cytokines including interferon gamma (IFN\(\gamma\)) and sLAG-3. Baseline tumor samples were collected prior to treatment and were analyzed by immunohistochemistry for LAG-3 (17B4), CD8 (C8/144B), and tumor cell programmed death-ligand 1 (PD-L1) expression (Agilent/Dako PD-L1 28-8 IHC pharmDx).\(^1-3\) Correlative analyses were performed.

Results Four weeks after treatment initiation, IFN\(\gamma\) levels increased over baseline in both treatment arms (table 1), but increases were significantly larger with NIVO+RELA than with NIVO (fold change 2.17 and 1.54, respectively; \(P<0.0001\)). Eight weeks after treatment initiation, sLAG-3 levels were modestly increased with NIVO (fold change 1.12; \(P<0.0001\)) and significantly decreased with NIVO+RELA (fold change -0.60; \(P<0.0001\)) (table 2), suggesting RELA-specific target engagement in the combination arm. No significant correlation was observed between baseline sLAG-3 and tissue-based LAG-3 expression in pretreatment tumors (Spearman’s \(r=0.21\)). In baseline tumor biopsies, LAG-3 showed moderate correlation with PD-L1 expression (\(r=0.53\)) (figure 1). The majority of LAG-3-negative tumors (<1\%) were also PD-L1 negative (<1\%). PD-L1 expression varied among LAG-3-expressing tumors, with differences observed in the range of 0–20% and 0–50% with LAG-3 and PD-L1 subgroups. CD8 levels correlated more strongly with LAG-3 than with PD-L1. Furthermore, patients whose tumors had higher LAG-3 expression ≥1% and ≥5% had numerically higher median progression-free survival and objective response rates in both the NIVO+RELA and NIVO arms.

Conclusions These exploratory analyses support the hypothesis that NIVO+RELA enhances immune activation compared with NIVO alone, and that LAG-3 may be a promising biomarker of tumor inflammation. Although LAG-3 alone may not be a useful predictive biomarker for selection of patients with melanoma for NIVO+RELA vs NIVO regimens, further work is required to assess the clinical utility of LAG-3, likely as part of a composite biomarker of immunotherapy susceptibility.

Ethics Approval All trial investigators received approval from their respective institutional review boards. The trial was conducted in accordance with the International Council for Harmonisation Good Clinical Practice guidelines, and all patients provided written informed consent before participation. An independent data monitoring committee provided oversight to assess the efficacy and safety profile of relatlimab and nivolumab.
Abstract 606 Figure 1  Correlation between LAG-3 and PD-L1 expression by immunohistochemistry.