Background
Tumor reliance on glycolysis is not only a hallmark of cancer, but also a mechanism of resistance to immunotherapy due to lactate-mediated immune suppression and competition for glucose between T cells and glycolytic tumor cells within the tumor microenvironment.\(^1,\)\(^2\) We have shown that CTLA-4 blockade is more effective in glycolysis-low tumors, or tumors lacking functional lactate dehydrogenase A (LDH-A), primarily due to functional destabilization of regulatory T cell suppression.\(^3\) LDH inhibitors (LDHi) have been reported to inhibit tumor glucose uptake and slow tumor cell proliferation in pre-clinical models of cancer.\(^4\) However, the optimal conditions for pharmacologic inhibition of LDH in combination with immunotherapy to maximize anti-tumor immune and therapeutic responses require further study.

Methods
B16F10-bearing C57BL/6 mice were treated daily with LDHi GNE-140 (oral gavage) and/or biweekly with anti-CTLA-4 (intraperitoneal injection) and tumor volume was measured twice per week. Bioactivity of LDHi was confirmed by quantification of lactate and LDH enzymatic activity in sera and tumors, as well as immunoblotting for LDHA protein levels. In separate experiments, tumors were harvested after 3 administrations of anti-CTLA-4 and processed for flow cytometric analysis of tumor-infiltrating lymphocytes to assess infiltration, activation, and function. Glucose uptake was assessed in tumor and T cells by fluorescent glucose analog GlucoseCy3.

Results
At baseline, tumor cells express higher levels of LDHA and consume more glucose than tumor-infiltrating T cells, creating a therapeutic window for tumor-specific targeting of glycolysis. Serum LDH and lactate levels correlate with primary tumor burden, as well as tumor LDH levels. LDHi relies on the adaptive immune system and the overexpression of tumor LDH to delay melanoma growth. Inhibiting LDH in combination with CTLA-4 blockade is more effective in controlling tumor progression compared to CTLA-4 blockade alone, and this combination promotes effector T cell infiltration and activation while functionally destabilizing regulatory T cells. Treatment with LDHi reduces tumor cell glucose uptake while facilitating increased glucose uptake by tumor-infiltrating T cells.

Conclusions
CTLA-4 blockade with LDHi enhances effector T cell function while impairing regulatory T cell suppression. LDH inhibition is an effective strategy to reduce tumor cell glucose uptake, therefore increasing tumor glucose availability and facilitating an increase in tumor-infiltrating T cell glucose uptake. Serum LDH may serve as a biomarker for clinical response to LDHi. This study provides a rationale for combining immune checkpoint blockade with inhibitors of glycolysis for patients with highly glycolytic cancers.

REFERENCES