BACKGROUND

• Although immune checkpoint inhibition targeting cytotoxic T lymphocyte antigen-4 (CTLA-4) and/or programmed cell death-1 (PD-1) has significantly improved survival outcomes for advanced melanoma patients, a large segment of the patient population does not respond or experiences recurrence after a relatively short duration of treatment.

• Resistance to mounting an effective antitumor immune response is multifactorial (Figure 1), and a multitude of combination approaches are being tested to address the problem.

• Entinostat (ENT) is an oral, class 1 selective histone deacetylase inhibitor that has been shown in a preclinical study to synergize with immune checkpoint blockade to inhibit tumor growth through down-regulation of regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs), functional immunosuppression, and alteration of an immune suppressive tumor microenvironment to one more favorable for antitumor immune response.

• Results from the ongoing ENCORE-601 study demonstrated that ENT combined with pembrolizumab (PEMBRO) elicited a 31% response rate in a cohort of 13 patients with melanoma progressing on or after a PD-1 blocking antibody.

• Correlational studies of pre- and posttreatment tissue and blood samples from patients treated with ENT plus PEMBRO have been conducted to confirm the mechanism of action of ENT in combination with immune checkpoint blockade and whether predictive markers of response can be identified.

METHODS

• ENCORE-601 employed a Simon 2-stage design to assess activity of ENT 5 mg QW PO, combined with PEMBRO 200 mg Q3W IV across 4 cohorts: 1. advanced anti–PD-(L)1-naive non-small cell lung cancer (NSCLC); 2. advanced NSCLC progressed on anti–PD-(L)1 treatment; 3. advanced melanoma progressed on anti–PD-(L)1 treatment; 4. advanced anti–PD-(L)1-naive microsatellite stable colorectal cancer

Key Exploratory Objectives

• To assess the ratio of effector T cells to Tregs in peripheral blood and tumor biopsies pre- and posttherapy.

• To evaluate inflammatory T-cell signature changes in peripheral blood and tumor biopsies pre- and posttherapy.

• To evaluate changes in number of MDSCs in peripheral blood and tumor biopsies pre- and posttherapy (flow cytometry).

• Pretreatment and posttreatment biomarker analysis included gene expression by a beta version of the PanCancer IQ 360™ assay (NanoString Technologies, Inc, Seattle, WA). PD-(L)1 expression by immunohistochemistry and levels of CD8+ T cells and LOX1+CD15+MDSCs by immunofluorescence staining on paraffin-embedded human tissue sections. Phenotypic evaluation of immune cell subsets was also conducted in peripheral blood samples collected pretreatment (cycle 2, day 1 (C2D1)) and posttreatment (C2D15) – FOR RESEARCH USE ONLY. Not for use in diagnostic procedures. NanoString is a registered trademark of NanoString Technologies, Inc., in the United States and/or other countries.

• Tumor Inflammation Signature (TIS) score was calculated as a linear combination of 18 genes that measure peripheral suppressed immune response and are normalized to 10 housekeeping genes previously identified to have stable expression across all cancer types.

RESULTS

Clinical Efficacy Summary

• Baseline demographic and prior therapy data are summarized in Table 1A and Table 1B.

• 4 patients (31%) had a partial response (PR; 3 confirmed, 1 unconfirmed), and 4 (31%) additional patients had stable disease (Figure 2).

• 4 patients continued on therapy with durable response/clinical benefit.

• Significant increase in TIS observed when pooled pre- and postdose samples were compared (Figure 3A), suggesting elevated tumor microenvironment inflammation.

• In a subset of patient matched samples, 2 of 3 responding patients showed a robust TIS increase (Figure 3B).

• After treatment, the tumor microenvironment becomes more supportive of T-cell activity (Figure 4A).

• IO 360 signature scores for IFN-γ (Figure 4B), CD8 T-cells (Figure 4C), and Cytotoxic Cells (Figure 4D) were elevated, suggesting enhanced support for treatment for cytotoxic T-cell activity in the tumor microenvironment.

• Intratumor CD8+ T cell and MDSC change is consistent with gene expression results and treatment effect noted in Figure 5 – CDB+ T cells and LOX+CD15+ MDSCs measured by immunofluorescence.

• CD8:MDSCs ratio increased in 6 of 8 (75%) patients (Includes 3 PRs and 1 ongoing SD), which may suggest clinical benefit.

• Increases in both T cells and MDSCs were observed only in those tissue samples converted from noninflamed to inflamed.

• Regardless of clinical outcome, a decrease in MDSCs (~35.7%; n=9) and an increase in CD8+ T cells (47.4%; n=9) between pre- and posttreatment biopsies were noted.

SUMMARY AND CONCLUSIONS

• ENT in combination with PEMBRO exhibits clinical efficacy in melanoma patients previously exposed to immune checkpoint inhibition.

• This combination has an acceptable toxicity profile.

• Preliminary biomarker analysis supports the hypothesis that the addition of ENT restores inflammation in the tumor microenvironment necessary for successful re-treatment with an anti–PD-(L)1.

REFERENCES