Entinostat Increases the Frequency of Tumor-Specific Effector T-cells and Their Functionality is Enhanced by Anti-OX40 Leading to Durable anti-Tumor Effects

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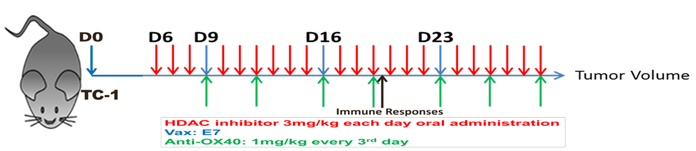
Background

The epigenetic deregulation of T-cells and enhanced numbers of immunosuppressive cells in the TME are associated with decreased anti-tumor effects. Hence, targeting the epigenetic modifications using modulators such as histone-deacetylase inhibitors (HDACi) provides the basis for a potential role for these agents in cancer immunotherapy. Entinostat, an HDACi has been shown to reprogram the TME by impacting the numbers of CD8 T-cells and immunosuppressive cells, resulting in enhanced anti-tumor activity when combined with immune-checkpoint blockade [1]. However, the combination effect of Entinostat with anti-OX40 remains poorly explored. Signaling through OX40 is known to enhance the effector functions of CD8 T-cells. In addition, it inhibits production of IL17a cells, which prevent apoptosis of MDSCs. However, as a single agent anti-OX40 has not yet shown promising results in the clinic. Therefore, we hypothesized that the combination of Entinostat with anti-OX40 will enhance the effector-functions of CD8 T-cells while simultaneously reducing the immunosuppressive cells including MDSCs in the TME, leading to improved anti-tumor effects.

Methods

In the TC-1 mouse tumor model, Entinostat (3 mg/kg) in combination with anti-OX40 (1 mg/kg) and tumor-specific vaccine (E7-peptide; 3 doses one-week apart) was given. Tumor growth and mice survival were recorded. Three days after the second immunization, immune-responses were determined in the tumors.



TC-1 cell line: Generated from murine lung epithelial cells immortalized with HPV16 E6 and E7 and h-ras.
HDAC inhibitor: Entinostat (3 mg/kg) through P.O. route.
Vaccine: E7 (CTL epitope, 100 µg/mouse) along with PADRE (aK-Cha-VAAWTLKAAa, where "a" is D alanine, and "Cha" is l-cyclohexylalanine), a small 13-mer non-natural pan HLA-DR-binding sequence that is a potent T cell epitope (T helper epitope, 20 µg/mouse-Peprotech) and QuilA (adjuvant, 10 µg/mouse-BioLegend) through S.C. route.
Anti-OX40: 1 mg/kg through I.P. route.

Results

Entinostat synergizes with anti-OX40 leading to complete tumor regression with tumor free survival of mice

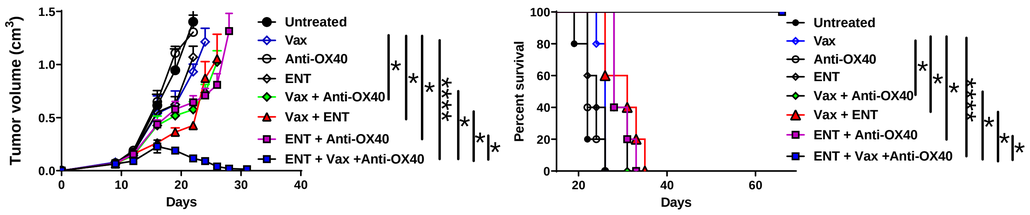


Fig. 1. Entinostat synergizes with anti-OX40 to regress tumor in 100% of the mice. C57BL/6 mice were treated as described and tumor growth and survival were determined. For growth, significance was determined by student's t-test, while for survival, significance was determined by Log-rank (Mantel-Cox) test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$ and ****$p \leq 0.0001$).

Entinostat synergizes with anti-OX40 to enhance total and antigen-specific granzyme B$^+$ and IFN-gamma$^+$ CD8$^+$ T-cells in the TME

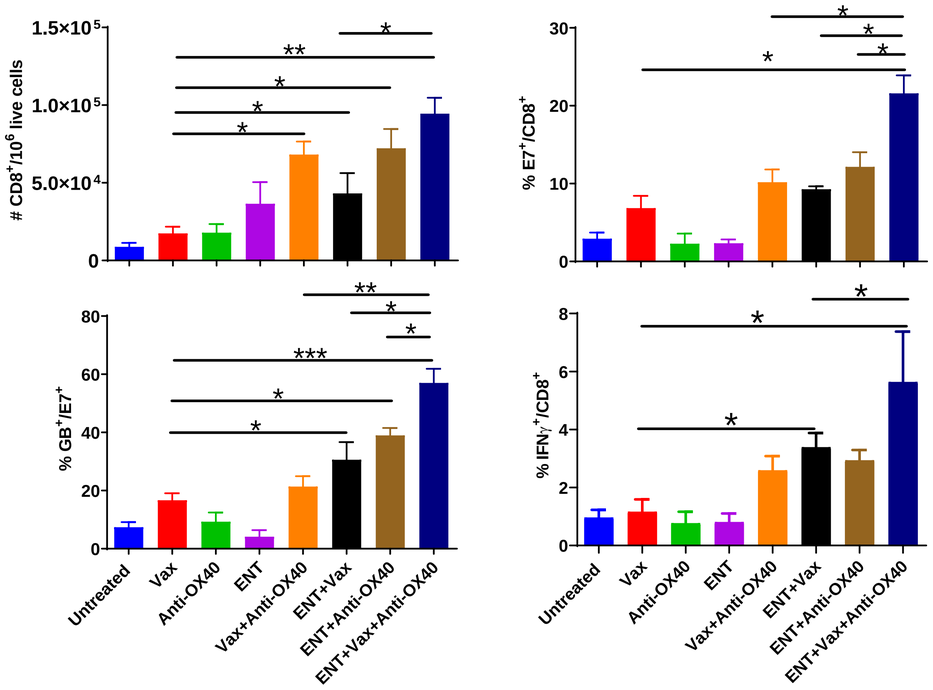


Fig. 2. Effects of Entinostat with anti-OX40 on the frequency and functionality of CD8$^+$ T cells in the TME. TC-1 tumor-bearing C57BL/6 mice were treated as described, except 3 days after the administration of the 2nd vaccine dose, mice were sacrificed and tumors harvested for assessment of immune response. One-tailed unpaired Student's *t* test was used to determine significance (*$p \leq 0.05$; **$p \leq 0.01$ and ***$p \leq 0.001$). GB = Granzyme B.

Entinostat with anti-OX40 reduces the IL17a producing CD4$^+$ and immunosuppressive cells in the TME

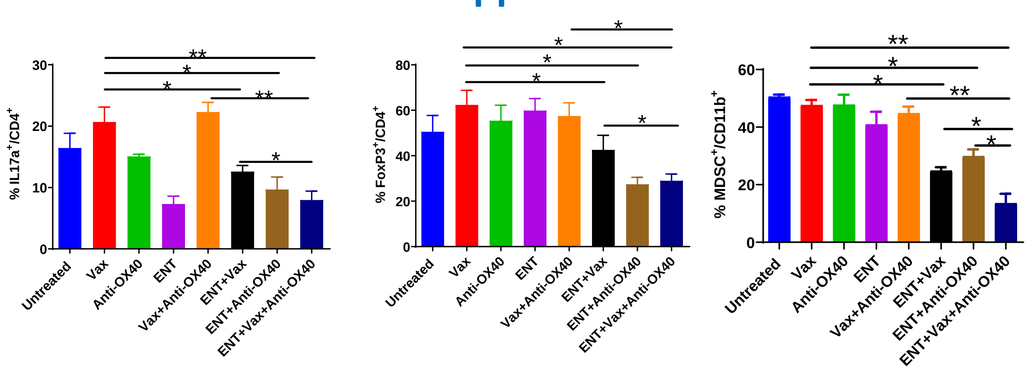


Fig. 3. Effects of Entinostat with anti-OX40 on the frequency of IL17a CD4 cells, Tregs and MDSCs in the TME. One-tailed unpaired Student's *t* test was used to determine significance (*$p \leq 0.05$ and **$p \leq 0.01$).

Entinostat with anti-OX40 enhances activation of macrophages

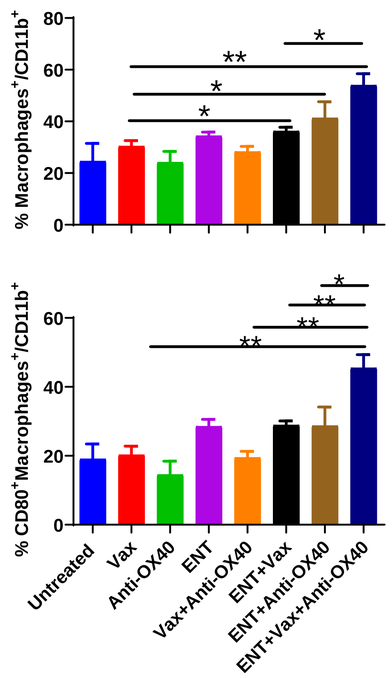


Fig. 4. Entinostat with anti-OX40 enhances activation of the macrophages in the TME. One-tailed unpaired Student's *t* test was used to determine significance (*$p \leq 0.05$ and **$p \leq 0.01$).

Summary and Conclusion

- These results highlight the ability of Entinostat with anti-OX40 in increasing the numbers of effector T-cells in the TME.
- Entinostat with anti-OX40 significantly enhanced the functionality of these tumor-infiltrated effector cells leading to induction of robust and durable anti-tumor responses.
- Importantly, Entinostat with anti-OX40 further decreased the numbers of immunosuppressive populations in the TME.
- In addition, Entinostat with anti-OX40 enhanced the activation of macrophages, which indicates enhanced antigenic presentation in the TME.
- These data highlight that anti-OX40 enhanced the anti-tumor efficacy of Entinostat, which can be a promising strategy for cancer-immunotherapy.

Reference

1. Terranova-Barberio M, et al., HDAC inhibition potentiates immunotherapy in triple negative breast cancer. Oncotarget. 2017; 8:114156-114172.

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