Enhanced Anti-tumor Activity of the Combination of Entinostat and NKTR-214 in Renal and Colon Cancer Tumor Models

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BACKGROUND
Combination strategies are required to improve outcomes for immunoresistant solid tumor therapies. Entinostat (NKT-214) is a class I selective histone deacetylase inhibitor that is under preclinical study to target immune-suppressive mechanisms in the tumor microenvironment to improve the efficacy of immune checkpoint blockade, vaccines and cytokines.1,2 NKTR-214 is a novel immunostimulatory melanoma vaccine.3 A phase 2 clinical trial of entinostat combined with high-dose interleukin 2 demonstrated enhanced clinical efficacy and a well tolerated safety profile in patients with advanced melanoma.4 Based on these data, the potential for combinational cell therapy activity, characterized by enhanced tumor antigen presentation in combination with immune checkpoint blockade, is attractive. In the current study, we investigated the therapeutic efficacy of entinostat and NKTR-214 in preclinical models of renal cell carcinoma and colon cancer.

MATERIALS AND METHODS
Cell Culture
The murine CT26 colon carcinoma cell line and RENCA renal cell carcinoma cell lines were maintained in vitro as a monolayer culture in RPMI-1640 medium supplemented with 10% fetal bovine serum and L-glutamine (2mM) at 37°C in an atmosphere of 5% CO2.

Tumor Inoculation and Drug Treatment
Female BALC3T3 mice (7-8 weeks) were used in this study. In the s.c. tumor model, each mouse was inoculated with 5 x 105 viable cells subcutaneously on the right flank (200 mm3 tumor volume target). Treatments were initiated when the mean tumor size reached approximately 140 mm3; treatments continued for 4 weeks. Mice were assigned to drug treatments as follows: Group 01: vehicle control, Group 02: entinostat 1 mg/kg, Group 03: NKTR-214 0.8 mg/kg, Group 04: entinostat 1 mg/kg + NKTR-214 0.8 mg/kg, Group 05: entinostat 5 mg/kg, Group 06: NKTR-214 0.8 mg/kg + entinostat 5 mg/kg, Group 07: entinostat 5 mg/kg + NKTR-214 0.8 mg/kg, Group 08: entinostat 5 mg/kg + NKTR-214 0.8 mg/kg + entinostat 5 mg/kg. All drug treatments were repeated weekly. The combination treatment also enhanced CD4 T cell effector function (CD45+CD3+CD4+INF-γ+) and CD8+ T cell effector functions (CD8+PD-1+). The combination treatment also reversed the MDSC increase. Mice (n=3) were treated with drugs as described in the materials and methods. On day 7 after drug treatment, the mice were harvested, and single cell suspensions were isolated, stained, and analyzed by FACS.

RESULTS

Figure 1. Combination of entinostat + NKTR-214 enhanced CD8+ T cell effector functions in the CT26 model

Figure 2. The combination treatment increased Th1 CD8 T cells in the RENCA model

Figure 3. Anti-tumor activity of entinostat in combination with NKTR-214 was observed in RENCA, a renal cell carcinoma model. 1. The Bartlett test was used to check homogeneity of variance and normality. 2. In each experiment, the same number of mice were used in all groups. 3. A 2-way ANOVA was used to compare group means. 4. Any group with only one observation, no statistical test was performed for that specific group.

CONCLUSIONS
• Combination of entinostat and NKTR-214 significantly inhibited tumor growth in CT26 and B16/F10 models.
• The combination greatly enhanced T cell cytokines by producing more INFγ and granzyme in all models.
• NKTR-214 alone or with higher dose entinostat also significantly decreased Tregs in CT26 model.
• Entrinostat alone increased M1DCs in the B16/F10 model, and the addition of NKTR-214 reversed the M1DC increase.
• Based on these preclinical data, entinostat and NKTR-214 is promising combination to be explored in patients with CRC or renal cell carcinoma.

References:

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