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

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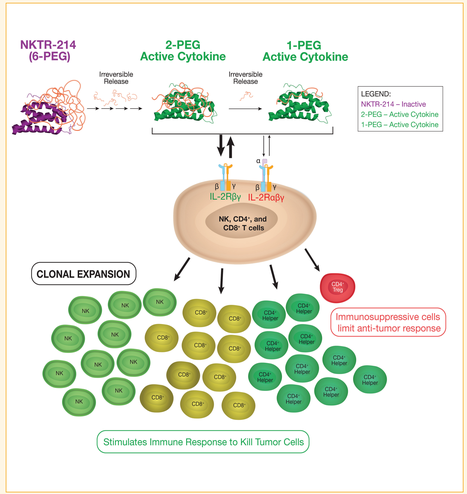
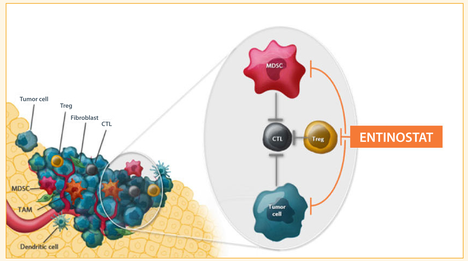
BACKGROUND

Combination strategies are required to improve outcomes for immune-mediated anti-tumor therapies. Entinostat is a class 1 selective histone deacetylase inhibitor shown in preclinical studies to target immune-suppressive mechanisms in the tumor microenvironment to improve the efficacy of immune checkpoint blockade, vaccines and cytokines[1-3]. NKTR-214 is a novel CD122-biased agonist with promising safety, tolerability and anti-tumor efficacy[4,5]. 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 renal cell cancer[6,7]. Based on these data and the potential for combinatorial anti-tumor activity, we investigated the anti-tumor efficacy and immune response of entinostat combined with NKTR-214 in CT26 colon cancer and RENCA renal cell cancer syngeneic tumor models.





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% CO_2.

Tumor Inoculation and Drug Treatment

Female BALB/c mice (7-8 weeks) were used in this study. In the s.c. tumor model, each mouse was inoculated subcutaneously at the right lower flank with tumor cells in 0.1 ml of PBS. Animals were randomized and then treated with vehicle, ENTINOSTAT, NKTR-214 alone or in combination when the mean tumor size reached approximately 140 mm^3.

The animals were dosed at 0.8 mg/kg with NKTR-214 intravenously once every 9 days for total 4 doses, and/or 1 mg/kg or 5 mg/kg of entinostat via oral gavage daily for 4 weeks.

Tumor volumes were measured twice weekly, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where a and b are the long and short diameters of the tumor, respectively. The entire procedures of dosing as well as tumor and body weight measurement were conducted in a Laminar Flow Cabinet.

Tumor Infiltrating Lymphocytes Analysis

Tumors were harvested on day 7 after drug treatment, cut into small pieces, and incubated in collagenase solution in a 37°C water bath for 2 hours. The dissociated tumors were passed through a 70-μm filter to generate single-cell suspensions. Cells were stimulated with Leukocyte activation cocktail with GolgiPlug for 6 hours, then stained with the following rat-anti-mouse antibodies CD45, CD3, CD4, CD8a, Foxp3, CD335, CD11b, F4-80, GR-1, PD-1, granzyme B, IFN-r, TNF-a and viability dye. Cells were then analyzed on a BD Fortessa or BD LSR II using FlowJo software.

Statistical Analysis for TILs

Flow cytometry data was further analyzed by R, an open source programming language. Statistical differences between each group were described using p-values. P-values were calculated in the following steps:

1. The Bartlett test was used to check homogeneity of variance and normality.
2. If the p-value of the Bartlett test was not less than 0.05, ANOVA and two sample t-test was used to compare group means.
3. If the p-value of the Bartlett test was less than 0.05, Kruskal-wallis test and Wilcoxon rank sum test were used to compare group means.
4. For all two group comparisons, all the p-values are nominal p-values without multiple comparison adjustment. For any group with only one observation, no statistical test was performed for that specific group.

RESULTS

Figure 1. **Entinostat and NKTR-214 combination led to tumor regression in CT26 model**

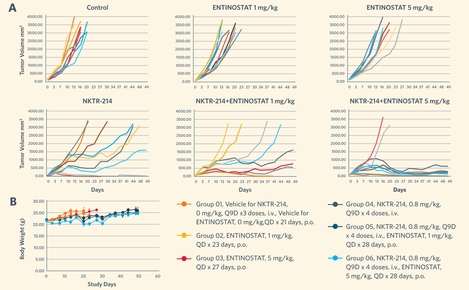


Figure. 1. Anti-tumor activity of entinostat in combination with NKTR-214 was observed in CT26, a syngeneic colon cancer mouse model. Mice were randomly grouped and the treatment started when the s.c. inoculated tumors reached 140 mm^3 (n=9 per group). Tumor volume **(Fig. 1A)** and body weight **(Fig. 1B)** was measure twice a week. No weight loss was observed in all animals.

RESULTS (continued)

Figure 2. **Combination of Entinostat + NKTR-214 enhanced CD8 T cell activation and cytotoxicity in CT26 model**

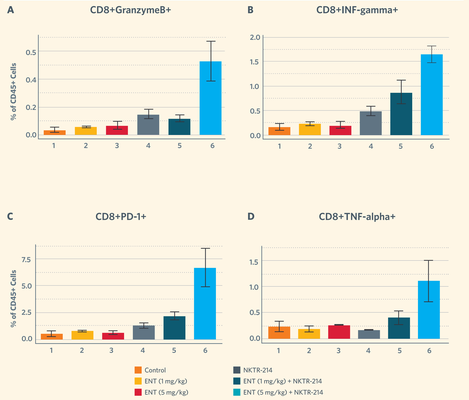


Figure 2. The combination treatment increased an activated Th1 CD8 T cell tumor microenvironment in the CT26 model. Mice (n=3) were treated with drugs as described in materials and methods. On day 7 after drug treatment, the tumors were harvested, and single-cell suspensions were stimulated, stained, and analyzed by FACS. Quantitative results are shown: the expression of Granzym B+ CD8 T cells (CD45+CD3+CD4-CD8+ GranzymB+) (A); INFγ+ CD8 T cells (CD45+CD3+CD4-CD8+ INFγ+) (B), PD-1+ CD8 T cells (CD45+CD3+CD4-CD8+ PD-1+) (C); and TNFα+ CD8 T cells (CD45+CD3+CD4-CD8+ TNFα+) (D).

Figure 3. **Combination of Entinostat + NKTR-214 reduced regulatory T cells in CT26 model**

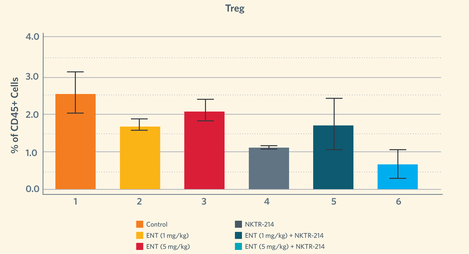


Figure 3. The combination treatment reduced Tregs (CD45+CD3+CD4+Foxp3+) in the tumor microenvironment in the CT26 model. Mice (n=3) were treated with drugs as described in the materials and methods. On day 7 after drug treatment, the tumors were harvested, and single-cell suspensions were stimulated, stained, and analyzed by FACS.

RESULTS (continued)

Figure 4. **Tumor regression after combination of entinostat and NKTR-214 treatment in RENCA model**

Figure 4. Anti-tumor activity of entinostat in combination with NKTR-214 was observed in RENCA, a syngeneic renal cell carcinoma mouse model. Mice were randomly grouped and the treatment started when the s.c. inoculated tumors reached 140 mm^3 (n=9 per group). Tumor volume **(Fig. 4A)** and body weight **(Fig. 4B)** were measure twice a week. The number of animals sacrificed due to cachexia were G3: n=5, G4: n=1, G5: n=1, G6: n=6.

Figure 5. **Combination of Entinostat + NKTR-214 enhanced CD8 T cell cytotoxicity in RENCA model**

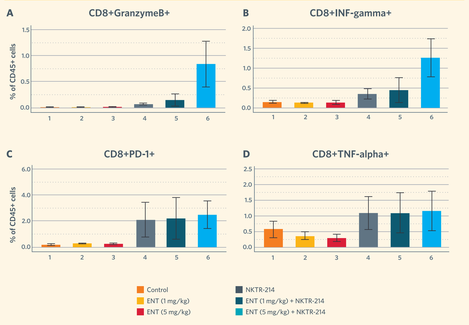


Figure 5. The combination treatment increased Th1 CD8 T cells in the tumor microenvironment in the RENCA model. Mice (n=3) were treated with drugs as described in materials and methods. On day 7 after drug treatment, the tumors were harvested, and single-cell suspensions were stimulated, stained, and analyzed by FACS. Quantitative results are shown: the expression of GranzymB+ CD8 T cells (CD45+CD3+CD4-CD8+GranzymB+) (A); INFγ+ CD8 T cells (CD45+CD3+CD4-CD8+ INFγ+) (B), PD-1+ CD8 T cells (CD45+CD3+CD4-CD8+ PD-1+) (C); and TNFα+ CD8 T cells (CD45+CD3+CD4-CD8+ TNFα+) (D).

RESULTS (continued)

Figure 6. **Combination of Entinostat + NKTR-214 significantly enhanced INFγ production in CD4 T cells in the RENCA model**

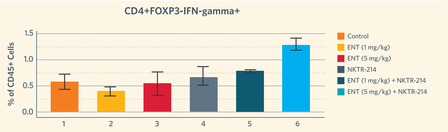


Figure 6. The combination treatment also enhanced CD4 T cell effector function (CD45+CD3+CD4+INFγ+) in the tumor microenvironment in the RENCA model. Mice (n=3) were treated with drugs as described in materials and methods. On day 7 after drug treatment, the tumors were harvested, and single-cell suspensions were stimulated, stained, and analyzed by FACS.

Figure 7. **Single agent entinostat increased MDSCs, however, adding NKTR-214 reversed MDSC increase in the RENCA model**

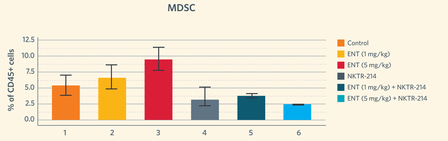


Figure 7. Single-agent entinostat increased MDSCs (CD45+CD3- CD11b+Gr-1+) in the RENCA model, however, adding NKTR-214 reversed this MDSC increase. Mice (n=3) were treated with drugs as described in materials and methods. On day 7 after drug treatment, the tumors were harvested, and single-cell suspensions were stimulated, stained, and analyzed by FACS.

CONCLUSIONS

- Combination of entinostat and NKTR-214 significantly inhibited tumor growth in CT26 and RENCA models.
- The combination greatly enhanced T-cell cytotoxicity by producing more IFNγ and granzyme B in both models.
- NKTR-214 alone or with higher dose entinostat also significantly decreased Tregs in CT26 model.
- Entinostat alone increased MDSCs in the RENCA model, and the addition of NKTR-214 reversed the MDSC increase.
- Based on these preclinical data, entinostat and NKTR-214 is a promising combination to explore in patients with CRC or renal cell carcinoma.

References:

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