The HDAC inhibitor HBI-8000 enhances immunotherapy with either PD-1 or PD-L1 blockade in the MC38 and 4T1 models of colon cancer

Reid P. Bissonnette, Alain Rolland, Bob Goodenow, Mireille Gillings. HUYA Bioscience International, San Diego, CA

Introduction & Rationale

Antibodies targeting the immune checkpoint receptors CTLA-4, PD-1 and its ligand PD-L1 have resulted in significantly improved clinical benefit in a growing number of cancer types. However, a significant number of patient's tumors are either innately resistant or develop resistance, and disease progresses. Therefore, rational drug combinations with checkpoint inhibitors and other immunomodulatory agents are imperative to improve the rate of durable responses and patient survival.

Histone deacetylase inhibitors (HDACi) are successful as monotherapy for hematologic malignancies, but to date have had limited clinical effectiveness against solid tumors. However, Class I selective HDACi have been clearly demonstrated to enhance anti-tumor immune response in multiple preclinical models through effects on immunogenicity, regulatory T cells, myeloid-derived suppressor cells (MDSCs), and effector cell functions.

HBI-8000 (chidamide) is a novel, orally bioavailable Class I (HDAC 1,2,3) and Class II (HDAC 10) selective HDACi with direct anti-tumor activity, and is approved for the treatment of relapsed/refractory PTCL (EPIDAZA, China). HBI-8000 has demonstrated positive effects on antitumor immunity, increasing the expression of NK and CTL receptors involved in tumor cell recognition, proteins functionally involved in effector cell activation and cell-mediated tumor cell lysis, thus enhancing the activity of CTL and NK cells. These data provided a strong rationale to explore combinations of HBI-8000 with antibodies targeting immune checkpoints.

METHODS

Reagents: Anti-PD-1 (clone RMP1-14) and anti-PDL-1 (clone 10F.9G2) were purchased from BioXcell. Antibody was dosed IP, biw x 3, and HBI-8000 PO by gavage, qd x 21.

Tumor Cells: MC38 murine colon carcinoma and 4T1 murine mammary carcinoma cells were maintained in DMEM or RPMI, respectively, with 10% FBS.

Mice and Tumors: Female C57BL/6 and BALB/c mice (Charles River) were 7-8 weeks old on Day 1. For MC38 tumor studies, mice were inoculated SC in the right flank with 1 x 10^6 cells in 0.1 mL. For 4T1 studies, mice were injected orthotopically (mammary fat pad) with 1 x 10^6 cells in 0.1 mL. When mean tumor growth reached 100–150 mm^3, the mice were randomized into the various treatment groups.

4T1 Lung Metastases: Metastatic foci were counted on D14, the last study day. Total counts were obtained by adding the number of foci in the superior, middle, inferior, and post-caval lobes of the right lung to the number counted in the left lung. Percent inhibition was defined as number of metastatic foci of the treated group divided by the metastatic foci of the control group x 100.

1st line PD-1/PD-L1 Failure and 2nd Line Therapy: In the MC38 model, mice treated 1st line with PD-1 or PD-L1 antibodies typically segregate into 3 groups; ≈20% show no response and rapidly progress. Similarly, ≈20 % experience complete tumor regression. The majority of animals experience slow tumor progression or stable disease, but develop resistance and rapidly progress, failing 1st line antibody therapy. Animals that met this criteria comprise the 2nd line efficacy study cohort and were placed sequentially into the six 2nd line efficacy groups.

RESULTS

Figure 1. HDACi HBI-8000 combined with PD-1 antibody produces statistically significant enhancement of anti-tumor and survival activities vs. single agents

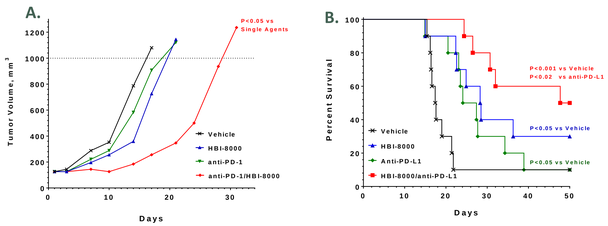
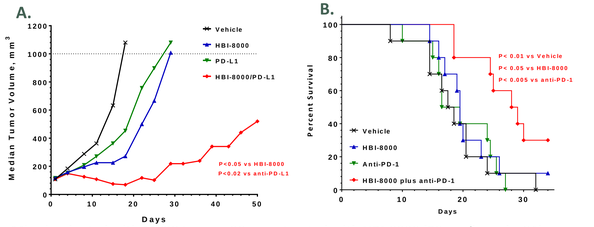
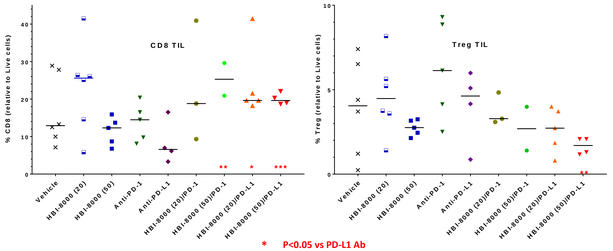


Figure 2. HDACi HBI-8000 combined with PD-L1 antibody produces statistically significant enhancement of anti-tumor and survival activities vs. single agents



Mice implanted with MC38 tumors SC were treated with HBI-8000 (50 mg/kg, qd x 21) or antibody (5 mg/kg, biw x 3) as indicated. Data shown in graphs is from representative study, and depicts Median Tumor Volume (A, C) and Kaplan-Meier Survival (B, D). Tumors were measured twice weekly. Statistical analysis performed using PRISM 7.00.

Figure 3. Combinations of HBI-8000 *plus* PD-1 or HBI-8000 *plus* PD-L1 antibodies significantly increase CD8+ and decrease Treg TIL in MC38 tumors



* P<0.05 vs PD-L1 Ab
** P<0.05 vs Single Agent
*** P<0.01 vs Single Agent

Mice treated with HBI-8000 (20 or 50 mg/kg, QD x 21) or antibody (5 mg/kg, BIW x 3) as indicated. Data shown from representative study. Statistical analysis (Unpaired t Test).

Figure 4. PD-L1 antibody combined with the HDACi HBI-8000 rescues mice with progressive PD-1 Ab-resistant MC38 tumor growth in a model of 1st line PD-1 antibody therapy failure

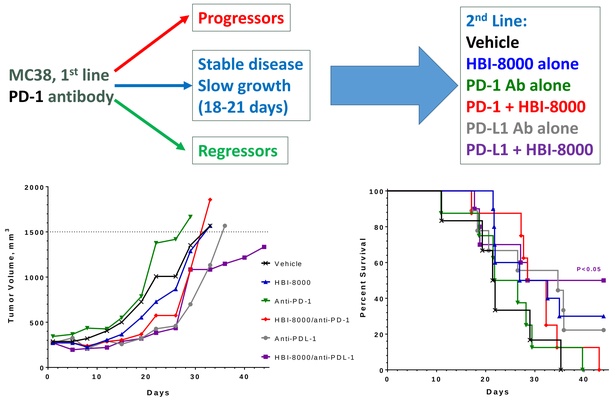
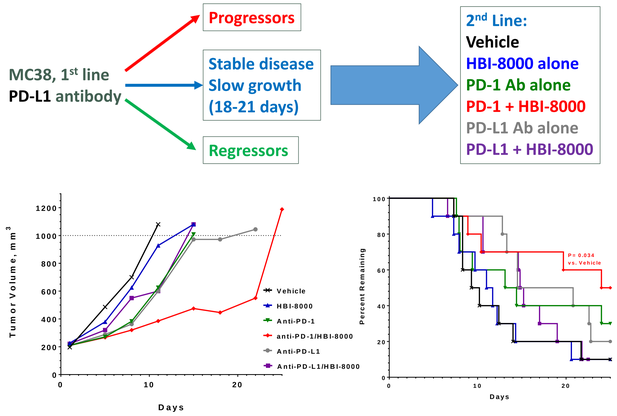
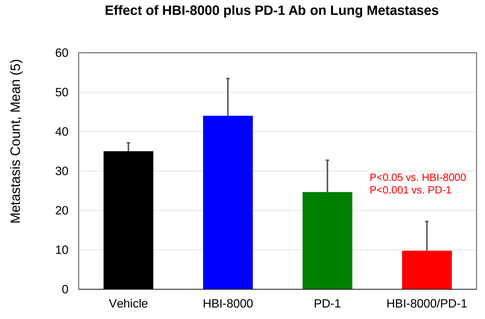


Figure 5. PD-1 antibody combined with the HDACi HBI-8000 rescues mice with progressive PD-L1 Ab-resistant MC38 tumor growth in a model of 1st line PD-L1 antibody therapy failure



Mice implanted with MC38 tumors SC were treated with 1st antibody (5 mg/kg, biw x 3) for 18-21 days, after which mice displaying stable or slow tumor growth were randomized into the six 2nd line treatment groups.

Figure 6. HDACi HBI-8000 combined with PD-1 antibody produces statistically significant reduction in lung metastases in the 4T1 TNBC model



Mice implanted orthotopically (mammary fat pad) with 4T1 tumors SC were as indicated for 18 days (nodule counts in LOOK SEE groups 50-75/lung), after which lungs were stained and foci counted using a dissecting microscope.

Summary and Conclusions

1. Combining the HDAC inhibitor HBI-8000 with checkpoint inhibitor antibodies (either PD-1 or PD-L1) in the MC38 model produced statistically significant increases in survival and delay in tumor progression compared to single agents
2. Tumor infiltrate (TIL) analysis revealed a statistically significant increase ($p<0.05$) in intratumoral CD8+ T cells (cytotoxic T cells) for both PD-1 and PD-L1
3. Other TIL did not achieve significance, however, combos showed a trend toward lower Tregs and granulocytic MDSCs
4. In MC38 tumor-bearing mice failing 1st line treatment with **PD-1 antibody**, 2nd line therapy with PD-L1 antibody combined with HBI-8000 showed a statistically significant benefit in survival, with single agent HBI-8000 and PD-L1 Ab trending
5. In MC38 tumor-bearing mice failing 1st line treatment with **PD-L1 antibody**, 2nd line therapy with PD-1 antibody combined with HBI-8000 showed a statistically significant benefit in survival, with single agent HBI-8000 and PD-1 Ab trending
6. In the 4T1 TNBC model of spontaneous metastasis, HBI-8000 combined with PD-1 Ab produced statistically significant and synergistic inhibition of metastasis
7. HUYA Bioscience has initiated a Phase 1b/2 study of nivolumab combined with HBI-8000 in Melanoma, Renal Cell Carcinoma and Non-Small Cell Lung Cancer (NCT02718066)