The β-carboline Harmine improves the therapeutic benefit of anti-PD1 in melanoma by increasing the MHC-I-dependent antigen presentation

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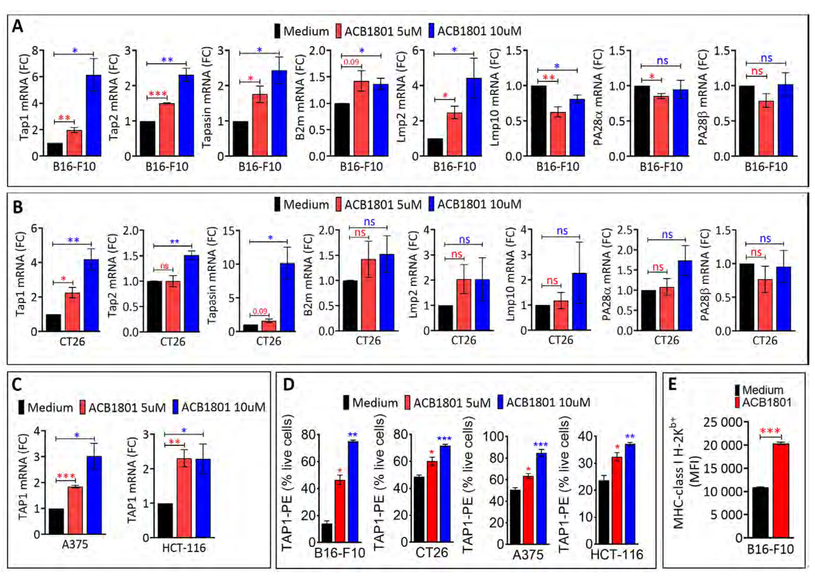
Abstract

Despite the success of anti-PD-1 therapy, many patients experience intrinsic or acquired resistance involving several non-mutually exclusive mechanisms. One of the most straightforward causes of the lack of responsiveness to anti-PD-1/PD-L1 is defects in the recognition of tumor cells by T cells, which can be related to the defects in the antigen presentation mechanism by MHC. Harmine, also referred to as ACB1801 molecule, is a dual-specificity tyrosine-regulated kinase 1A (DYRK1A) inhibitor that displays a number of biological and pharmacological properties. Here, we show that ACB1801 upregulates the mRNA expression of several proteins of the MHC-I such as Transporter Associated with antigen Processing TAP1 and 2, Tapasin and Lmp2 (hereafter referred to as MHC-I signature) in several cancer cells. Treatment of mice bearing melanoma B16-F10 with ACB1801 inhibits the growth and weight of tumors and induces a profound modification of the tumor immune landscape. Strikingly, combining ACB1801 with anti-PD1 significantly improves its therapeutic benefit in B16-F10 melanoma-bearing mice. These results suggest that, by increasing the MHC-I, ACB1801 can be combined with anti-PD1/PD-L1 therapy to improve the survival benefit in cancer patients displaying a defect in MHC-I expression.

Results:

1. ACB1801 increases the expression of several proteins of the MHC-I in various murine and human cancer cells

A and B: The mRNA expression of Tap1, Tap2, Tapasin, B2m, Lmp2, Lmp10, PA28α, PA28β, in melanoma B16-F10 (A) and colorectal CT26 (B) cancer cells. Cells were treated for 24 h with control or two concentrations of ACB1801. Results are reported as fold change (FC) relative to control cells treated with medium (black bars).



C: The mRNA expression of TAP1 in human melanoma A375 and colorectal HCT-116 Cells treated as indicated in A and B.

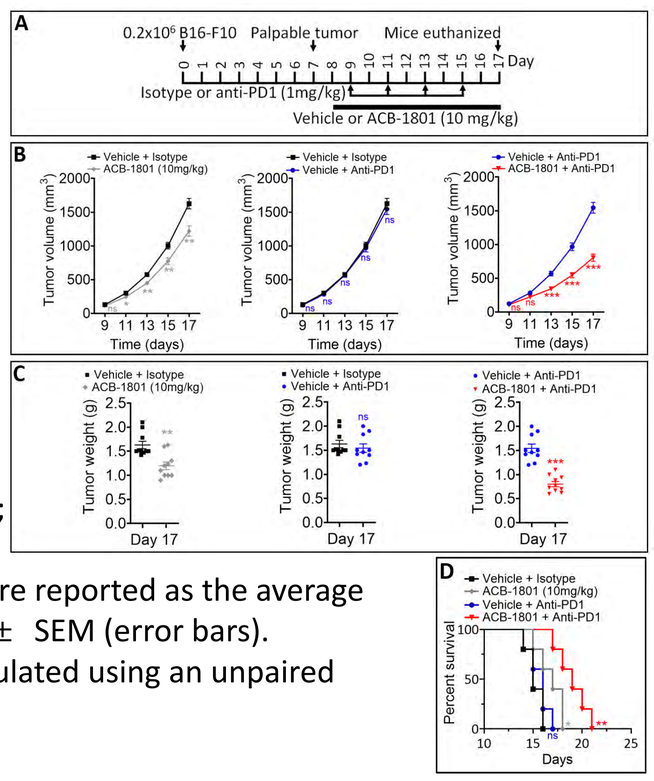
D: The expression of TAP1 protein in cells treated as indicated in A and B. Results are reported as % of positive cells relative to live cells.

E: The expression of MHC-class I H-2Kb allotype on the cell surface of B16-F10 cells treated for 24 h with control or ACB1801 (5 uM). Results are the averages of three independent experiments and are shown as mean ± SEM (error bars).

2. ACB1801 inhibits B16-F10 melanoma tumor growth and improves the therapeutic benefit of anti-PD-1

A: Experimental schedule of B16-F10 melanoma treatment with mono and combination therapies of ACB1801 and/or anti-PD-1.

B, C and D: Tumor growth curves (B), weight (g) at day 17 (C), and mice survival (D) of B16-F10 melanoma in mice treated with: vehicle and isotype (vehicle + isotype); isotype and 10 mg/kg ACB1801 (ACB-1801 10 mg/kg); vehicle and anti-PD-1 (vehicle + anti-PD-1); and a combination of ACB1801 and anti-PD-1 (ACB1801 + anti-PD-1). Results are reported as the average of 10 mice per group and shown as mean ± SEM (error bars). Statistically significant differences are calculated using an unpaired two-tailed student's t-test.



3. Treatment of B16-F10 tumor-bearing mice with ACB-1801 increases the infiltration of cytotoxic immune cells into the tumor microenvironment.

A: The percent (%) of indicated lymphoid cells infiltrating B16-F10 tumors treated as described in 2.

B: The ratio of CD8/Treg reported as percent (%) of cells infiltrating B16-F10 tumors treated as described in 2.

C: The percent (%) of indicated myeloid cells infiltrating B16-F10 tumors treated as described 2.

D and E: The percent (%) of live CD69+ (D), PD-1+ (E) NK cells, CD4+ effector T cells, and CD8+ T cells infiltrating B16-F10 tumors treated as described in 2. Data are reported as the average of 5 mice per group as mean ± SEM (error bars). Statistically significant differences are calculated in comparison to vehicle-treated tumors using an unpaired two-tailed student's t-test.

4. Retrospective analysis of the therapeutic value of MHC-I signature (TAP1, TAP2, TAPBP and LMP2) upregulation in melanoma patient cohorts.

A: The expression of MHC-I signature (TAP1, TAP2, TAPBP and LMP2) reported as FPKM in metastatic melanoma patients who are not responsive (NR) or responsive (R) to anti-PD-1.

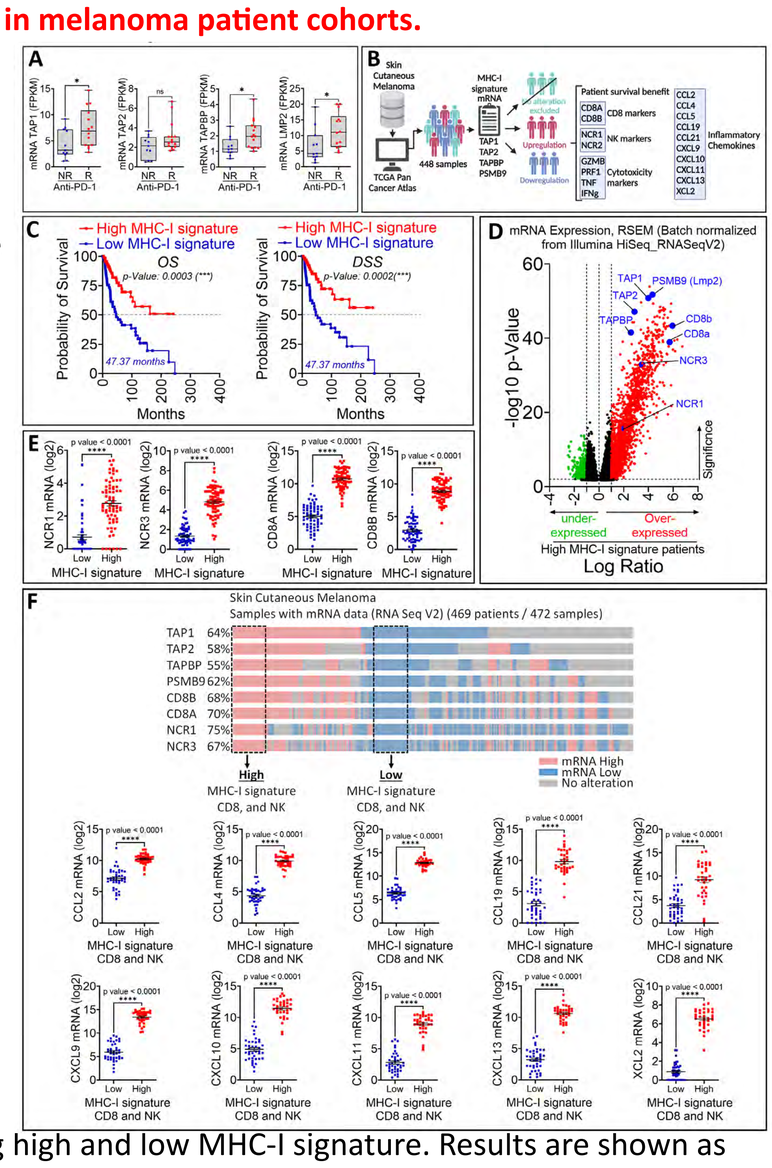
B: Workflow used for analyzing melanoma patient data in TCGA database.

C: Kaplan-Meier overall survival (OS, left panels) and disease-specific survival (DSS, right panels) curves of melanoma patients expressing high and low mRNA expression of MHC-I signature.

D: Volcano plot of over- and under-expressed genes in melanoma patients with high MHC-I signature.

E: The mRNA expression of NK markers (NCR1 and NCR3) and CD8 markers (CD8A and CD8B) in melanoma patients displaying high and low MHC-I signature. Results are shown as mean ± SEM (error bars). Statistically significant differences of high MHC-I signature are calculated compared to patients with low MHC-I signature using an unpaired two-tailed student's t-test (**** = p<0.0001).

F: *Upper panel:* Strategy used to extract melanoma patient data in TCGA database expressing low and high TAP1, TAP2, Tapasin (TAPBP), Lmp2 (PSMB9), CD8A, CD8A and NCR1 and NCR3 genes. Doted boxes define patients that we have considered to assess the expression of CCL2, CCL4, CCL5, CCL19, CCL21, CXCL9, CXCL10, CXCL11, CXCL13 and XCL2 chemokines. *Lower panel:* The mRNA expression of CCL2, CCL4, CCL5, CCL19, CCL21, CXCL9, CXCL10, CXCL11, CXCL13 and XCL2 in patients defined in the upper panel.



Conclusions

- ❑ β-carboline derivative ACB1801 potentiates the therapeutic benefit of anti-PD-1 in a B16-F10 melanoma mouse model, reported to resist to anti-PD-1 therapy and to express low levels of MHC-I.
- ❑ ACB1801 increases the expression of several proteins of the MHC-I such as TAP1, TAP2, TAPBP, and the low-molecular-weight protein 2 (LMP2).
- ❑ ACB1801 induces a deep modification of the immune landscape of B16-F10 tumors characterized by an increase of NK, CD4, and CD8 T cells and decrease of Tregs infiltration in the tumor microenvironment.
- ❑ The precise mechanism by which ACB-1801 increases the antigen presentation to MHC-I is still not fully understood.

The relevance of our study is underscored by clinical data showing that about 50% of cancer patients displayed an abnormal antigen presentation. Therefore, combining ACB1801 could substantially increase the number of cancer patients that would benefit from the impressive therapeutic value of ICI.

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