p300/CBP bromodomain inhibitor CCS1477 enhances the efficacy of immune checkpoint blockade therapy in cancer treatment

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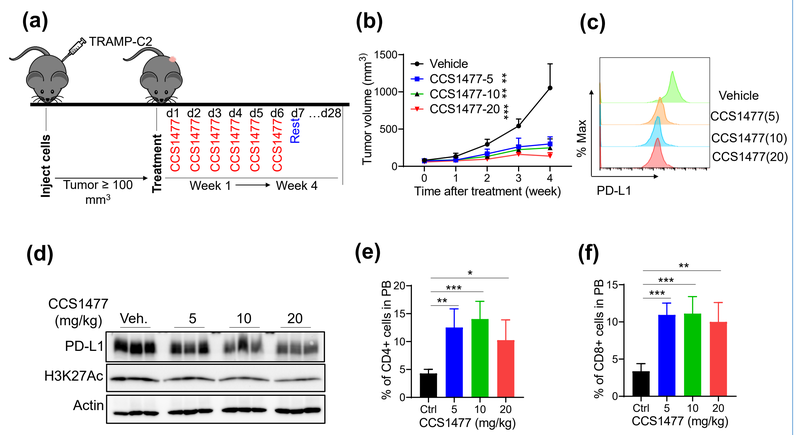
Background

Blockade of programmed death-ligand 1 (PD-L1) by therapeutic antibodies has shown promise as a strategy to treat cancer, yet clinical response in many types of cancer is limited. Tumor cells secrete PD-L1 through exosomes or splice variants, which has been described as a new mechanism for the resistance to PD-L1 blockade therapy in multiple cancers.

Our previous findings showed that the histone acetyltransferases p300/CBP could be recruited to the promoter of CD274 (encoding PD-L1) to regulate the expression of PD-L1. P300/CBP inhibition abrogated this process and reduced the secretion of exosomal PD-L1 by blocking the transcription of CD274, which combined with the anti-PD-L1 antibody to reactivate T cells function for tumor attack. In this study we have tested the outcome of combined immune checkpoint inhibitors with CCS1477 (inobrodib), a novel p300/CBP bromodomain inhibitor, in pre-clinical models of prostate cancer (PCa) and melanoma.

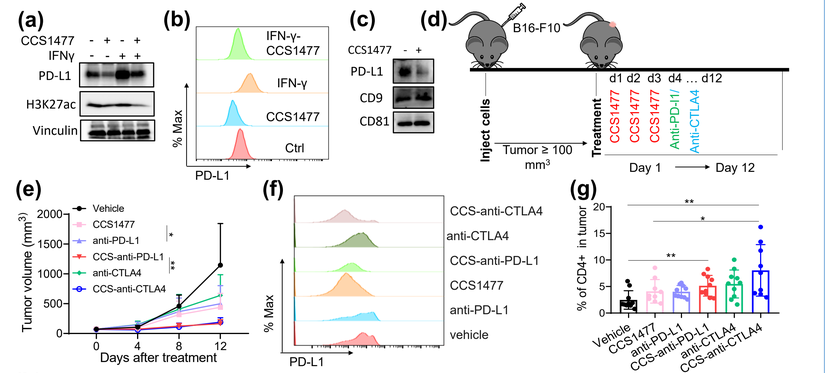
Results

CCS1477 decreases PCa PD-L1 expression *in vivo*



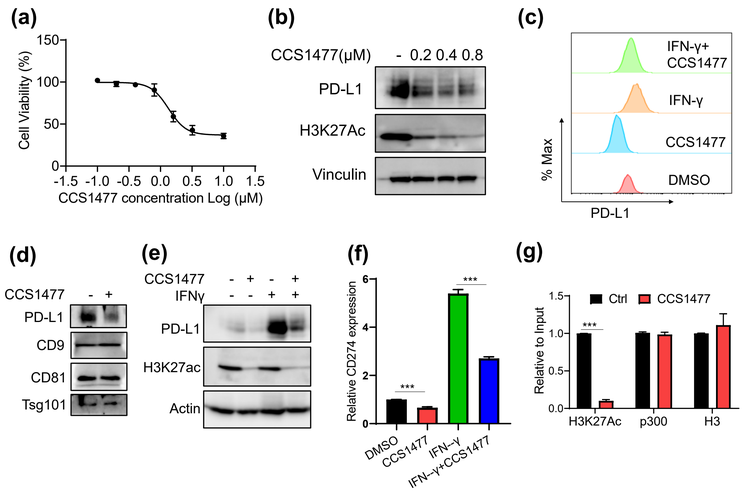
(a) A schema showing the experiment design of *in vivo* TRAMP-C2 xenograft and treatment strategy. TRAMP-C2 cells were injected into the flanks of C57BL/6 male mice. When the tumors reached 100 mm^3, mice were treated with vehicle or different concentrations of CCS1477 (5 mg/kg, 10 mg/kg, or 20 mg/kg by oral gavage daily, 6 days on, and 1 day off for 4 weeks). Tumor volumes (b) were shown as mean, PD-L1 expression was determined by flow cytometry assay (c) or western blot (d), CD4+ T cell population (e) and CD8+ T cell population (f) in peripheral blood (PB) was determined by flow cytometry assay and quantified.

CCS1477 enhances PD-L1 or CTLA4 blockade therapy in melanoma



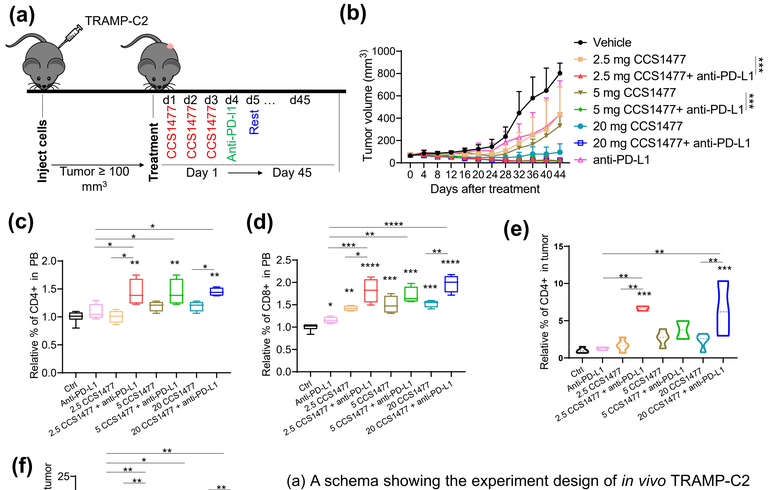
(a,b) A375 cells were treated with 10 ng/ml IFN-γ, 1 μM CCS1477 or combination for 24 hrs and then subjected to western blot (a) or FACS (b) to determine PD-L1 level. (c) A375 cells were treated with 1 μM CCS1477 for 24 hrs, harvest media for exosomal PD-L1 determination with western blot. (d) A schema showing the experiment design of *in vivo* B16-F10 xenograft and treatment strategy. B16-F10 cells were injected into the flanks of C57BL/6 female mice. When the tumors reached 100 mm^3, mice were treated with 5 mg/kg CCS1477, 200 μg anti-PD-L1 or anti-CTLA4 per mouse, or combination with CCS1477 for 12 days. Tumor volumes (e) were shown as mean, PD-L1 expression was determined by flow cytometry assay (f), CD4+ T cell population (g) and CD8+ T cell population (h) in tumors were determined by flow cytometry assay and quantified.

CCS1477 decreases PCa PD-L1 expression *in vitro*



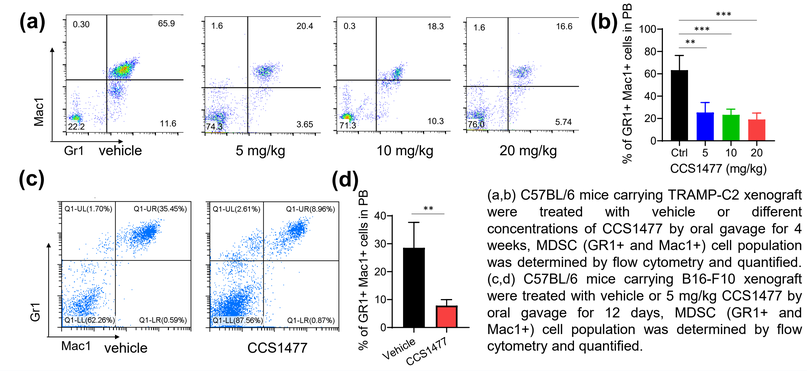
(a, b) DU145 cells were treated as indicated for 72 hrs and then subjected to MTT assay to determine cell viability (a) or western blot to determine the indicated proteins level (b). (c, e) DU145 cells were treated with 10ng/ml IFN-γ, 1 μM CCS1477 or combination for 24 hrs and then subjected to fluorescence activated cell sorting (FACS) (c) or western blot (e) to determine PD-L1 level. (d) DU145 cells were treated with 1 μM CCS1477 for 24 hrs and then harvest media for exosomal PD-L1 determination with western blot. (f) DU145 cells were treated with 10 ng/ml IFN-γ, 1 μM CCS1477 or combination for 24 hrs and then subjected to real-time q-PCR to determine the CD274 expression. (g) ChIP-qPCR analysis of H3K27Ac, p300 and H3 binding at CD274 enhancer in DU145 cells treated with 1 μM CCS1477 for 24 hrs.

CCS1477 enhances PD-L1 blockade therapy in PCa



(a) A schema showing the experiment design of *in vivo* TRAMP-C2 xenograft and treatment strategy. TRAMP-C2 cells were injected into the flanks of C57BL/6 male mice. When the tumors reached 100 mm^3, mice were treated with vehicle, different concentrations of CCS1477 (2.5 mg/kg, 5 mg/kg, or 20 mg/kg by oral gavage as indicated), 200 μg anti-PD-L1 per mouse or the combination for 45 days. Tumor volumes (b) were shown as mean +/-SEM, CD4+ T cell population in PB (c) or tumor (e), CD8+ T cell population in PB (d) or tumor (f) were determined by flow cytometry assay and quantified.

CCS1477 decreases MDSC population *in vivo*



(a,b) C57BL/6 mice carrying TRAMP-C2 xenograft were treated with vehicle or different concentrations of CCS1477 by oral gavage for 4 weeks, MDSC (GR1+ and Mac1+) cell population was determined by flow cytometry and quantified. (c,d) C57BL/6 mice carrying B16-F10 xenograft were treated with vehicle or 5 mg/kg CCS1477 by oral gavage for 12 days, MDSC (GR1+ and Mac1+) cell population was determined by flow cytometry and quantified.

Summary

CCS1477 (inobrodib) is active as monotherapy and enhances the efficacy of immune checkpoint blockade therapy in cancer treatment through decreasing PD-L1 expression and MDSC population.