CXCR4 Inhibition Modulates Tumor Microenvironment and Robustly Inhibits Growth of B16-OVA Melanoma

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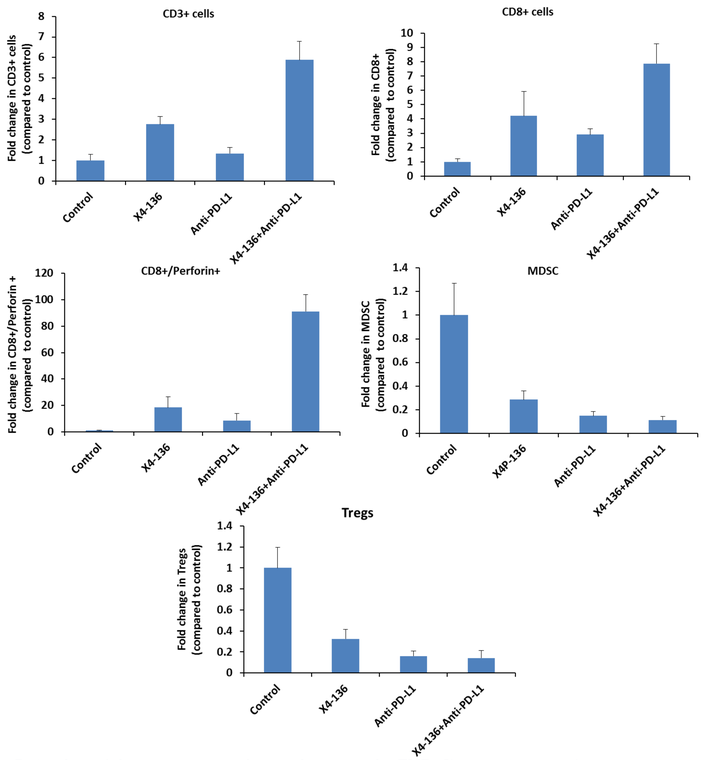
Background

The chemokine receptor CXCR4 is expressed on a range of immune cells. Additionally, CXCR4 has been found to be over-expressed in a variety of cancers and promotes cancer cell proliferation and metastasis, possibly by activating pro-survival signals that render cancer cells resistant to immune attack. Blockade of immune inhibitory pathways is emerging as an important therapeutic approach for the treatment of cancer. In our previous studies, X4-136, a CXCR4 antagonist, alone and in combination with various immune checkpoint inhibitors exhibited potent anti-tumor activity in the B16-OVA murine melanoma model. We report here results from additional in vivo studies as well as in vitro mechanistic experiments to determine the impact of CXCR4 inhibition on tumor cell gene expression and on immune-phenotypes within the tumor microenvironment.

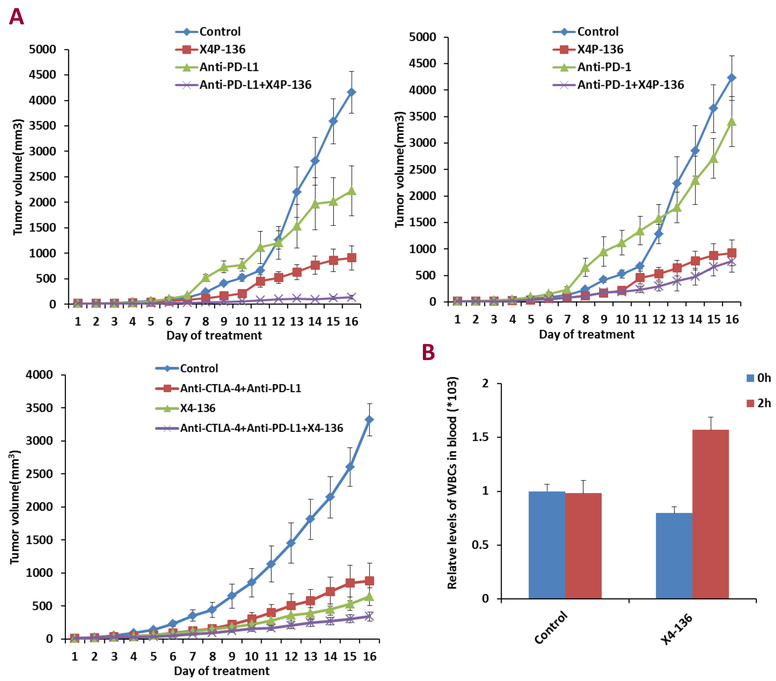
Results

Modulation of Immune-Phenotype in Tumor Micro-Environment



Reduction of immune-suppressive environment in TME. Single cell suspensions were prepared from tumor tissues by treating with collagenase and analyzed for various immune cell populations using flow cytometry.

Robust Inhibition of B16 Melanoma Growth by X4-136 as Single Agent and in Combination



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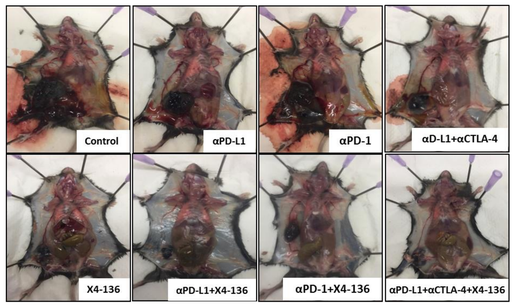
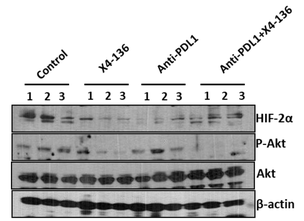


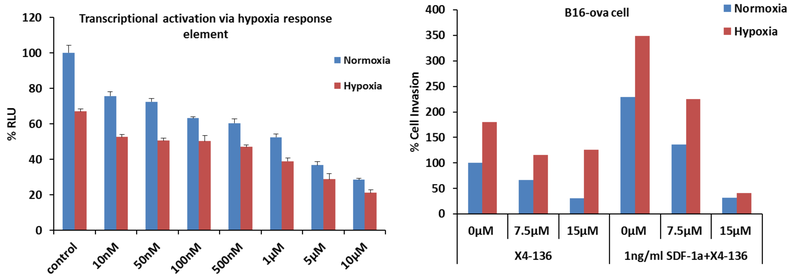
X4-136 either alone or in combination with Anti-PD-L1, Anti-PD-1 or Anti-PD-L1+ Anti-CTLA-4 antibodies inhibit the growth of B16-ova melanoma. $1*10^5$ B16-ova cells were implanted subcutaneously in C57BL/6 mice. After tumors attained a size of approx. 3*3 mm, mice were randomly grouped and treated for 16 days with vehicle (5-days on/ 2-days off), X4-136 (100 mg/kg, 5-days on/2-day off), Anti-PD-L1 (100µg/mouse every alternate day), Anti-PD-1 (100µg/mouse every alternate day), Anti-PD-L1+Anti-CTLA-4 (100µg/mouse every fourth day), Anti-PD-L1+X4-136, Anti-PD-1+X4-136 and Anti-PD-L1+ Anti-CTLA-4+X4-136. All treatments were well tolerated. (A) Tumor growth curve. (B) X4-136 increases WBC count in peripheral blood. Mice with B16-ova tumors were injected with vehicle or 100mg/kg of X4-136. White blood cells (WBC) were counted pre-dose (0h) and 2h post dosing. (C) Representative images of tumors in each treatment group

Inhibition of HIF-2α expression and Akt activation



Western Blot Analysis of Tumor Lysates. Tumor tissues were collected , flash frozen in liquid nitrogen and lysates were prepared. Immunoblotting was done for the expression of different proteins.

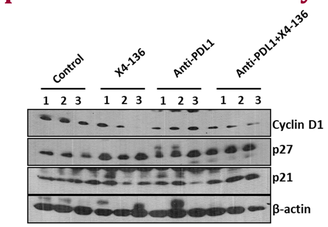
Suppression of HIF-2α Activity and Inhibition and Invasion



X4-136 inhibits transcriptional activation via HIF-2α response element. B16-ova in normoxic and hyopxic conditions were transiently transfected with pHRE-luc and pRL-luc and incubated with different concentrations of X4-136 for 24h. luciferase activity was measured using dual luciferase assay kit.

X4-136 inhibits the invasion of B16-ova cells through matrigel membrane. Transwell matrigel invasion chambers were used to assess the effect of X4-136 on B16-ova cell invasion.

Induction of p21, p27 and Reduction of Cyclin D1 Expression



Western Blot Analysis of Tumor Lysates. Tumor tissues were collected , flash frozen in liquid nitrogen and lysates were prepared. Immunoblotting was done for the expression of different proteins.

Conclusion

- X4-136 alone increased tumor infiltrating CD8+ T-cells and exhibited potent anti-tumor activity in the B16-OVA murine melanoma model.
- The enhanced anti-tumor activity was observed when X4-136 was added to anti-PD-L1 treatment. Addition of anti-CTLA-4 to combination of X4-136 and anti-PD-L1 did not significantly increase anti-tumor activity.
- The anti-tumor activities were associated with the reduction of immuno-suppressive MDSCs and Treg populations and the increase in immuno-stimulatory CD8+/Perforin+ cells in the tumor microenvironment.