Efficacy and Mechanism of Action of CXCR4 Inhibition in B16 OVA Melanoma Model

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Background

The chemokine receptor CXCR-4 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 cell 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, we demonstrated that combination of a CXCR4 antagonist with an anti-angiogenesis agent axitinib relieved myeloid-derived suppressor cell (MDSC) mediated immunosuppression and suppressed HIF-2α expression, which resulted in synergistic antitumor effects in 786-0 and A498 RCC xenografts. To further study the CXCR4 mechanism of action in an immune-proficient background, we investigated the activity of a CXCR4 antagonist (X4-136) in a syngeneic mouse tumor model. As the current murine models for RCC do not share the same genetic alterations with the human disease, the B16-OVA model was selected for this study. The CXCR4 antagonist was tested in combination with axitinib or with checkpoint inhibitors which are part of the standard of care in managing melanoma in clinic.

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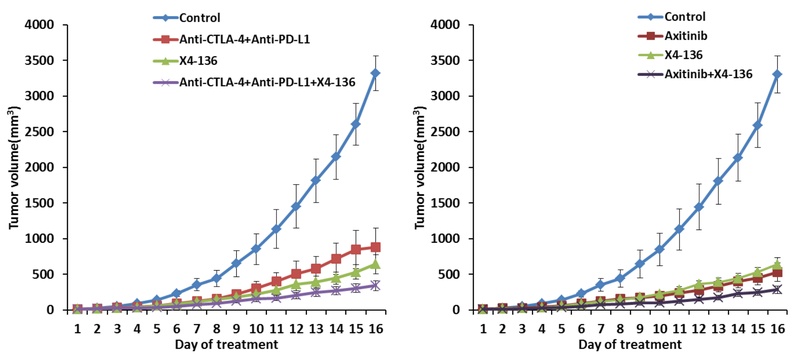
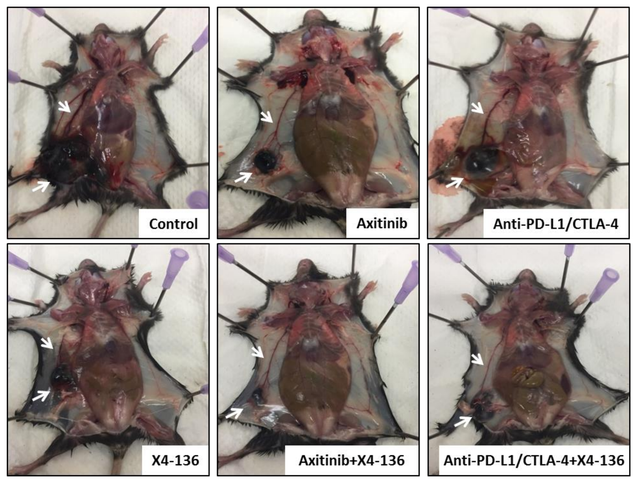
Results

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

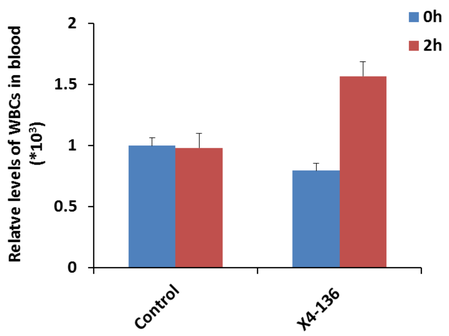
A



B

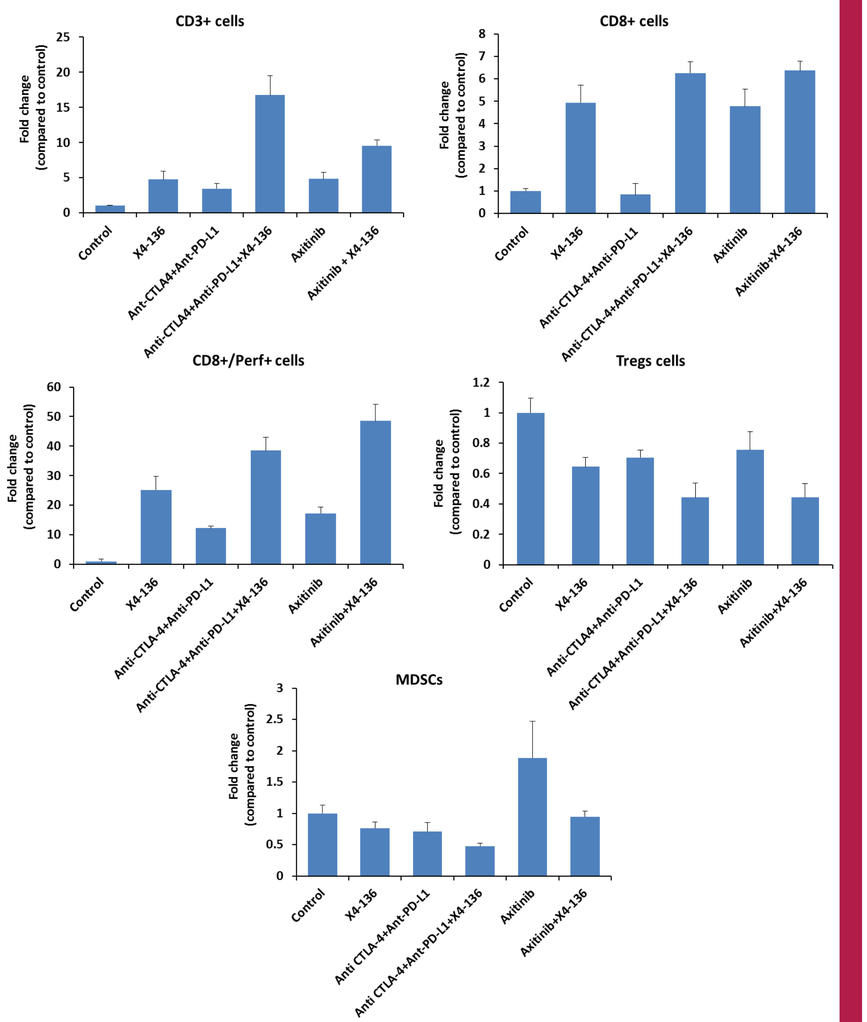


X4-136 either alone or in combination with Axitinib or Anti-PD-L1+ Anti-CTLA-4 antibodies inhibit the growth of B16-ova melanoma. $1*10^5$ B16-ova cells were implanted subcutaneously in C57BL/6 mice. After tumors attained a size of approx. 3*3 mm, mice were randomly grouped and treated for 16 days with vehicle (5-days on/ 2-days off), X4-136 (100 mg/kg, 5-days on/2-day off), Axitinib (30 mg/kg, 5-days on/2-day off), Anti-PD-L1 (100μg/mouse every alternate day) +Anti-CTLA-4 (100μg/mouse every fourth day), or axitinib+X4-136 and Anti-αPD-L1+ Anti-CTLA-4+X4-136. All treatments were well tolerated. (A) Tumor growth curve. (B) Representative images of tumors in each treatment group.



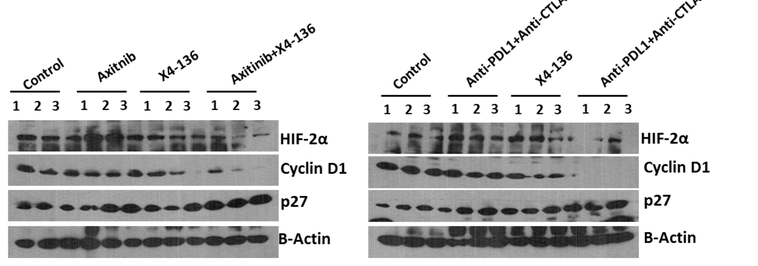
X4-136 increases WBC count in peripheral blood. Mice with B16-ova tumors were injected with vehicle or 100mg/kg of X4-136. White blood cells (WBC) were counted pre-dose (0h) and 2h post dosing.

Modulation of Immune-Phenotype in Tumor Micro-Environment



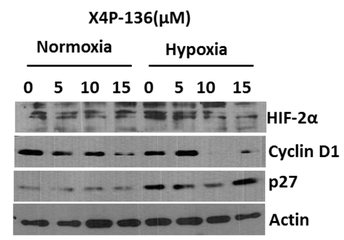
Reduction of immune-suppressive environment in TME. Single cell suspensions were prepared from tumor tissues by treating with collagenase and analyzed for various immune cell populations using flow cytometry.

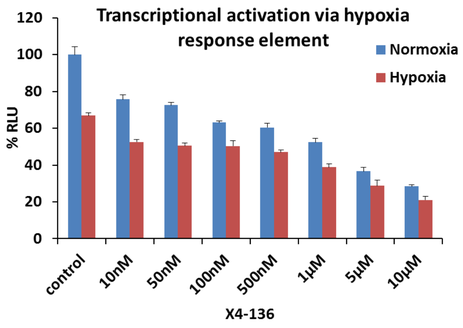
Western Blot Analysis of Tumor Lysates: Induction of p27and Reduction of Cyclin D1 Expression



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.

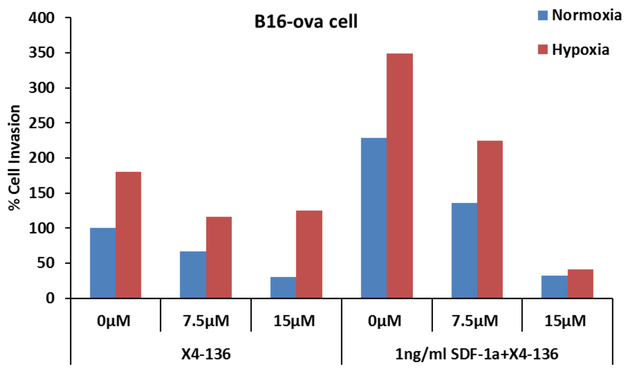
Suppression of HIF-2α Activity and Inhibition of Cyclin D1 and Invasion





Reduction of cyclin D1 and induction of p27 by X4-136. B16-ova cells in normoxia and hypoxia were treated with X4P-136 for 48h. Immunoblotting was done for the expression of different proteins.

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.

Conclusion

- X4-136 alone potently inhibited growth of the B16-Ova murine melanoma.
- Enhanced activity was observed when X4-136 was added to either axitinib or to anti-CTLA-4/PD-L1 treatments.
- 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.
- X4-136 reduces the invasive capability of B16-ova cells as well.