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

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

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. *p<0.05 B16 cells were implanted subcutaneously in C57BL/6 mice. After tumors attained a size of approx. 5-10 mm, mice were randomly treated and treated for 16 days with vehicle (5 days on/2 days off), X4-136 (100 mg/kg), 5 days on/2 days off). Anti-PD-1 (100µg/mouse every alternate day), Anti-PD-L1 Anti-CTLA-4 (100µg/mouse every fourth day). Anti-PD-L1-X4-136 (100µg/mouse every alternate day). Anti-PD-L1 Anti-CTLA-4 (100µg/mouse every alternate day). Anti-PD-L1 Anti-CTLA-4 (100µg/mouse every alternate day).

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 immunosuppressive MDSCs and Treg populations and the increase in immunostimulatory CD8+/Perforin+ cells in the tumor microenvironment.

Inhibition of HIF-2α expression and Akt activation

Suppression of HIF-2α Activity and Inhibition and 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.

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