Abstract Control # 374

Intratumoral CD40 activation and checkpoint blockade induces systemic anti-melanoma immunity that eradicates disseminated tumors

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Abstract

Although surgical resection is a reliable treatment for localized melanoma, treatment options for metastatic melanoma are limited. Melanoma Brain metastasis (MBM) is a major clinical problem in patients with advanced melanoma, and the incidence of brain metastasis is increasing every year. Thus, there is a significant unmet need for effective therapies for metastatic melanoma and MBM patients.

Agonistic CD40 antibodies generate strong tumor specific CD8 T cell response and anti-tumor activity; however systemic anti-CD40 therapy has been associated with cytokine release syndrome and liver toxicity.

We studied the anti-melanoma activity and mechanism of action of a non-replicating adenovirus encoding a CD40-targeting chimeric immunostimulatory protein (ISF35) by local intratumoral delivery approach to treat local and distant melanoma.

Mice bearing established B16 melanomas on both right and left flanks or on right flank and in brain were treated intratumorally with ISF35 or rAd.empty to the right flank tumor and received anti-PD1 plus anti-CTLA-4 systemically. Anti-tumor effects of mono or combination therapies were determined by mice survival and tumor growth measurement. The mechanistic contribution of immune cells to this therapy was determined by using antibody blockades. Immune cell infiltrates in tumor and expression of activation markers on these cells were analyzed by flow cytometry.

Intratumoral administration of ISF35 generates systemic anti-tumor immunity mediates by CD8 T cells and suppress both injected and distant uninjected wild-type B16.F10 melanomas. However, tumors did not completely regress after therapy. When combined with checkpoint inhibitors, ISF35 generates synergistic systemic anti-melanoma immunity that eradicates both injected and uninjected distant subcutaneous melanoma and uninjected brain melanoma with 40% of mice cured. The systemic anti-tumor activity of ISF35/anti-PD-1/anti-CTLA-4 was associated with greater production of melanoma-specific CD8 T cells with an activated phenotype.

Immunotherapy based on intratumoral CD40 activation is potentiated by PD-1 and CTLA-4 blockade and this combination generates functional and long-lasting anti-tumor CD8 T cell immunity that systemically suppresses melanoma metastases. This approach will be a better option for treatment of patients with metastatic melanoma that does not respond to checkpoint blockage monotherapy.

ISF35 Structure

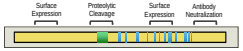
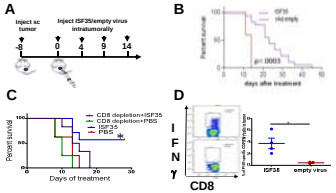


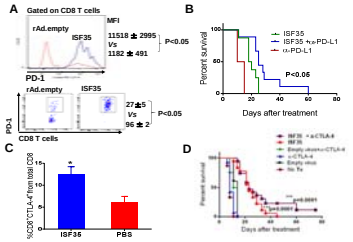
Diagram of the ISF35 transgene (recombinant CD40 ligand) with domain mapping that effect expression and immunogenicity

Anti-tumor Activity of ISF35 Monotherapy



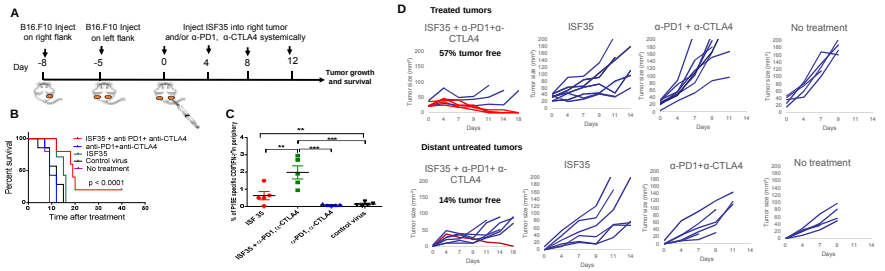
Anti-tumor activity of ISF35. Mice bearing sub cutaneous B16.F10 tumor (500,000 cells/tumor) were treated intratumorally. (A) Treatment strategy (B) mice survival (C) CD8 T cell-depleted mice survival and (D) tumor antigen T cell activation. Tumor infiltrating lymphocytes (TIL) were isolated from mechanically disrupted tumors by lymphocytes separation medium and cultured with P15E peptide for 6 hrs before performing CD8T cell and IFNg staining. Percent of $CD8^+IFNg^+$ cells (left) and cumulative data (right). Data is representative of at least 2 independent experiments and analyzed by unpaired two-tailed t test. *p<0.05. Error bars are SEM. Survival analysis was performed with the log-rank test

Synergistic Effect of ISF35 and Checkpoint Blockade



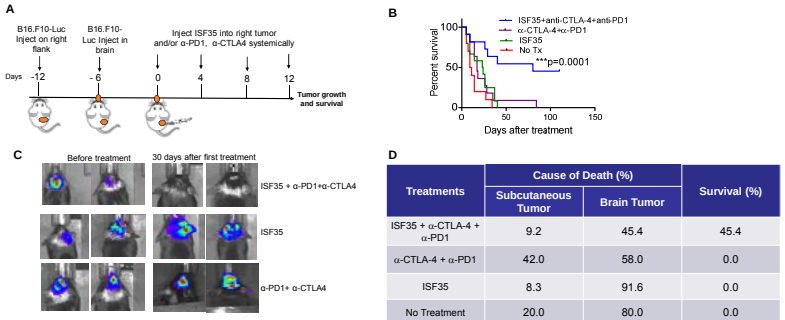
Efficacy of combination therapy. Mice bearing subcutaneous B16-F10 were intratumorally treated with ISF35, control virus, or PBS, leukocytes were stained after 6 days of treatment for the presence of $CD45^+CD8^+PD\text{-}1^+$or $CD8^+CTLA4^+$. MFI (mean fluorescence intensity) upregulation of (A) PD-1 and (C) CTLA-4 on tumor-associated CD8 T. Mice survival after systemic (B) anti- PD-L1 or (D) anti-CTLA-4 and/or intratumoral ISF35 treatment Data is analyzed by unpaired two-tailed t test. *p<0.05. Error bars are SEM. Survival analysis was performed with the log-rank test

ISF35, anti-CTLA-4, and anti-PD-1 Synergize to Reject Local Treated and Distant Untreated Tumors



ISF35, anti-CTLA-4, and anti-PD-1 synergize to reject local and distant tumors and generate systemic immunity. (A) Treatment strategy (B) mice survival (C) tumor antigen (p15E) specific CD8 T cells in circulation and (D) growth of treated and distant B16.F10 tumors. Data is representative of at least 2 independent experiments and analyzed by unpaired two-tailed t test. *p<0.05. Error bars are SEM. Survival analysis was performed with the log-rank test

ISF35, anti-CTLA-4, and anti-PD-1 Synergize to Reject Local Treated and Untreated Brain Melanoma



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Treatments	Cause of Death (%)		Survival (%)
	Subcutaneous Tumor	Brain Tumor	
ISF35 + α-CTLA-4 + α-PD1	9.2	45.4	45.4
α-CTLA-4 + α-PD1	42.0	58.0	0.0
ISF35	8.3	91.6	0.0
No Treatment	20.0	80.0	0.0

ISF35, anti-CTLA-4, and anti-PD-1 synergize to reject local treated tumors and distant untreated brain tumors. (A) Treatment strategy (B) mice survival (C) representative luminescence images from treated animals and (D) cause of mouse death (subcutaneous or brain tumor) and survival summary. Data is representative of at least 2 independent experiments. Survival analysis was performed with the log-rank test

Conclusions

- Intratumoral treatment with ISF35 combined with both anti-CTLA-4 and anti-PD-1 synergize to reject local treated tumors and distant untreated brain melanoma.
- ISF35 synergizes with anti-CTLA-4 and anti-PD-1 blockade to cure more than 40% of mice and develop long term immune memory.
- Intratumoral treatment with ISF35 induces robust expansion of tumor-specific CD8 T cells, resulting in tumor suppression and prolonged survival of mice.
- ISF35 combined with both anti-CTLA-4 and anti-PD-1 generates synergistic systemic anti-melanoma immunity that eradicates both injected and non-injected distant melanoma.

References

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