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Induction of systemic immunity through single-site intratumoral CD40 activation and checkpoint blockade eradicates melanoma in the brain

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Abstract

Background: Melanoma brain metastases (MBM) are a rapidly growing clinical problem with up to 60% of melanoma patients developing MBM over the course of their disease. Locoregional treatment with surgery, radiotherapy, radiosurgery, and newer drug classes such as checkpoint inhibitors and targeted agents against BRAF-mutation melanoma have shown limited effectiveness against MBM (Lancet 2015; 16: e486-97). MBM patients continue to have poor clinical outcomes and life expectancies of only 3-7 months. Thus, there is a significant unmet need for effective therapies for MBM patients. Direct intratumoral administration of a non-replicating adenovirus encoding a CD40-targeting chimeric immunostimulatory protein (ISF35) leads to generation of potent melanoma-specific T cells. When combined with checkpoint inhibitors, ISF35 generates synergistic systemic anti-melanoma immunity that eradicates both injected and uninjected distant melanoma with 40% of mice cured using a B16 model. Based on this evidence of systemic activity, we hypothesized that ISF35/checkpoint inhibitors combination treatment may have systemic activity against MBM.

Methods: A MBM mouse model was developed using luciferase-expressing B16 (B16-Luc). B16-Luc cells were subcutaneously (s.c.) implanted in the right flank of C57BL/6 mice 12 days prior to treatment. B16-Luc cells were then injected into the brain six days prior to treatment. Following tumor establishment at both sites, ISF35 was injected intratumorally in the right flank tumor on days 0, 4, 8, and 12. Simultaneously, anti-PD1 and anti-CTLA-4 antibodies were systemically administered.

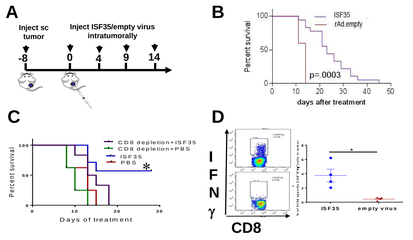
Results: Intratumoral administration of ISF35 in combination with anti-PD-1 and anti-CTLA-4 significantly increased ($p<0.01$) mouse survival compared to untreated mice. Median survival was not reached for the ISF35 plus checkpoint combination, was 10 days for untreated mice, 24 days for ISF35 monotherapy, and 17 days for anti-PD-1/anti-CTLA-4 combination therapy. ISF35/anti-PD-1/anti-CTLA-4 combination treatment of s.c. tumors resulted in complete and durable abscopal regression of brain tumors as assessed by bioluminescent imaging 30 days following initiation of treatment. In contrast, brain melanoma tumors continued to grow in the ISF35 monotherapy or anti-PD-1/anti-CTLA-4 treatment groups. 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, including upregulated PD-1 surface expression.

Conclusions: These results suggest ISF35 may improve the effectiveness of checkpoint inhibitors therapy for metastatic melanoma, including in the brain.

ISF35 Structure

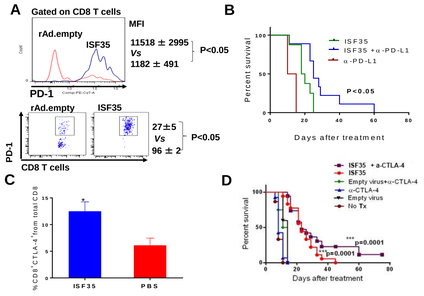
Diagram of the ISF35 transgene (recombinant CD40 ligand) with domain mapping that effect expression and immunogenicity

Anti-tumor Activity of ISF35 Monotherapy



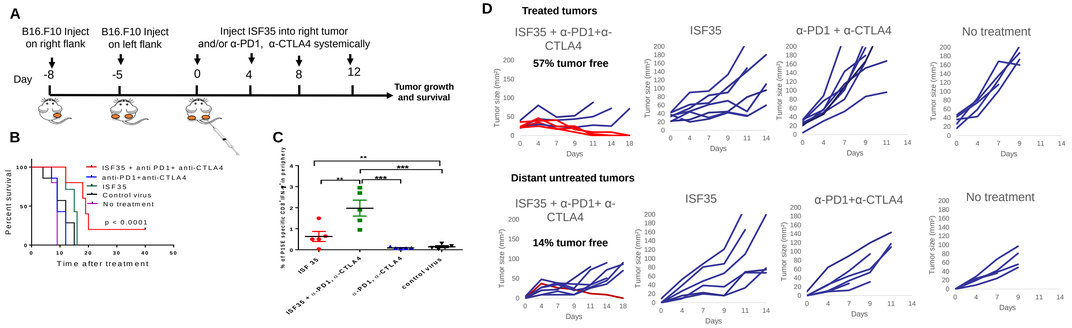
Anti-tumor activity of ISF35. Mice bearing sub cutaneous B16.F10 tumor (500,000 cells/tumor) were treated intratumorally. (A) Treatment strategy (B) mice survival (C) CD8 T cell-depleted mice survival and (D) tumor antigen T cell activation. Tumor infiltrating lymphocytes (TIL) were isolated from mechanically 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



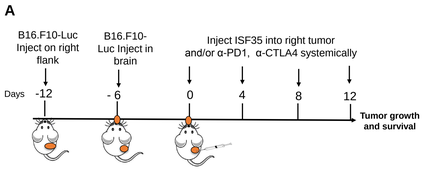
Efficacy of combination therapy. Mice bearing subcutaneous B16-F10 were intratumorally treated with ISF35, control virus, or PBS, leukocytes were stained after 6 days of treatment for the presence of CD45$^+$CD8$^+$PD-1$^+$or CD8$^+$CTLA4$^+$. MFI (mean fluorescence intensity) upregulation of (A) PD-1 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

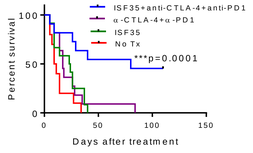
ISF35, anti-CTLA-4, and anti-PD-1 Synergize to Reject Local Treated and Distant Untreated Tumors

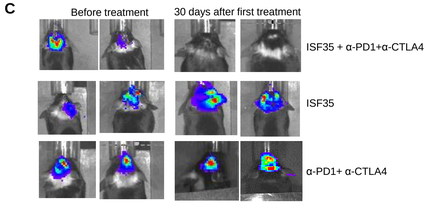


ISF35, anti-CTLA-4, and anti-PD-1 synergize to reject local and distant tumors and generate systemic immunity. (A) Treatment strategy (B) mice survival (C) tumor antigen (p15E) specific CD8 T cells in circulation and (D) growth of treated and distant B16.F10 tumors. Data is representative of at least 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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