Induction of potent systemic anti-melanoma immunity through intratumoral CD40 activation and checkpoint blockade

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1. SUMMARY

Purpose: 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 recombinant adenovirus expressing a stabilized version of CD40L (rAd.CD40L) by local intratumoral delivery approach to treat metastatic melanoma.

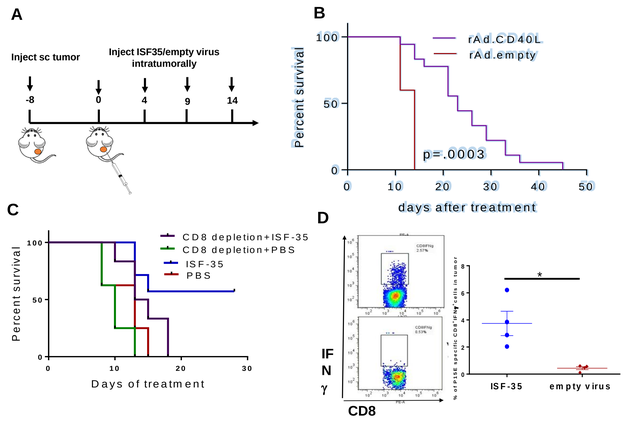
Experimental Design: Mice bearing established B16 melanomas were treated intratumorally with rAd.CD40L (ISF35) or rAd5 control virus and received anti-PD1 plus anti-CTLA-4 systemically. Anti-tumor effects of mono or combination therapies were determined by mice survival and tumor growth measurement. The mechanistic contribution of immune cells to this therapy was determined by using antibody blockades. Immune cell infiltrates in tumor and expression of negative regulators on these cells were analyzed by flow cytometry.

Results: Intratumoral administration of rAd.CD40L generated systemic anti-tumor immunity mediated by CD8 T cells and suppressed both injected and distant uninjected wild-type B16.F10 melanomas. However, tumors did not completely regress after therapy. Analysis of tumor-infiltrating leukocytes revealed that almost 100% of tumor-infiltrating CD8 T cells in the rAd.CD40L-treated group had up-regulation of the T cell inhibitory molecule PD-1. Combined treatment with rAd.CD40L plus anti-PD1 was highly synergistic and induced higher numbers of melanoma specific CD8 T cells systemically. Concomitant CTLA-4 blockade further improved the efficacy of treatment and led to complete tumor regression of tumor in about 50% of mice and generated memory CD8 T cells response.

Conclusion: 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. These results suggest combination of rAd.CD40L with checkpoint blockade inhibitors may offer a promising immunotherapeutic option of metastatic melanoma that does not respond to checkpoint blockade therapy.

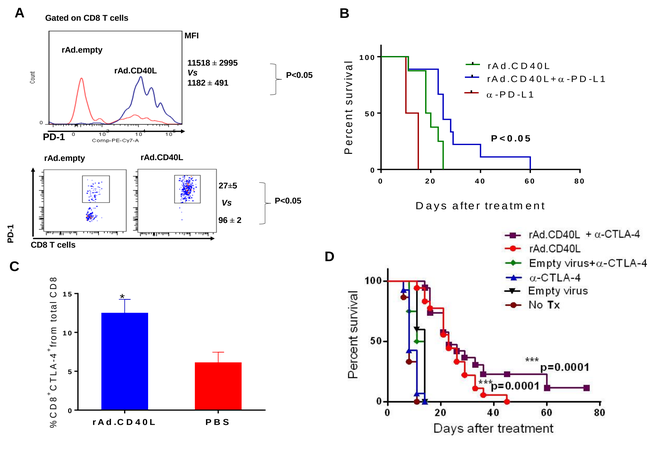
2. METHODS AND RESULTS

Anti-tumor Activity of Monotherapy



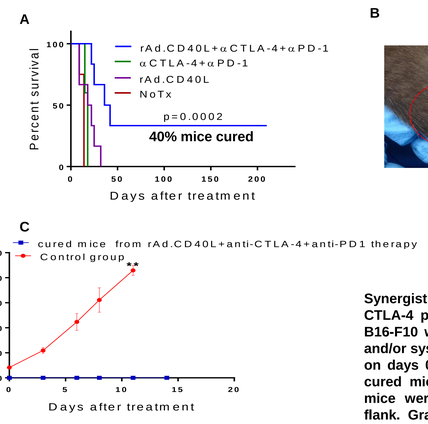
Anti-tumor activity of rAd.CD40L. Mice bearing subcutaneous B16.F10 tumor (500,000 cells/tumor) were treated intratumorally: (A) treatment strategy (B) mice survival (C) CD8 T cell-depleted mice survival and (D) tumor infiltrating lymphocytes (TIL) were isolated from mechanically disrupted tumors by lymphocyte separation medium and cultured with P15E peptide for 6 hrs before performing CD8 T 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

Efficacy of rAd.CD40L + checkpoint blockade



Efficacy of combination therapy: Mice bearing subcutaneous B16-F10 were intratumorally treated with rAd.CD40L or empty virus/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 cells. 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

Synergistic effect of rAd.CD40L plus anti-CTLA-4 plus anti-PD1 therapy



Synergistic effect of triple combination (rAd.CD40L plus anti-CTLA-4 plus anti-PD1) therapy. Mice bearing subcutaneous B16-F10 were treated intratumorally with rAd.CD40L (ISF35) and/or systemically with anti-CTLA-4 plus anti-PD1 antibodies on days 0, 4, 9 and 14: (A) mice survival after therapy (B) cured mice developed vitiligo at tumor site and (C) cured mice were re-challenged with B16.F10 tumor at opposite flank. Graph depicts tumor growth at various time points. 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

rAd.CD40L and anti-CTLA-4/anti-PD-1 checkpoint blockades synergize to reject local and distant tumors and generate systemic immunity:

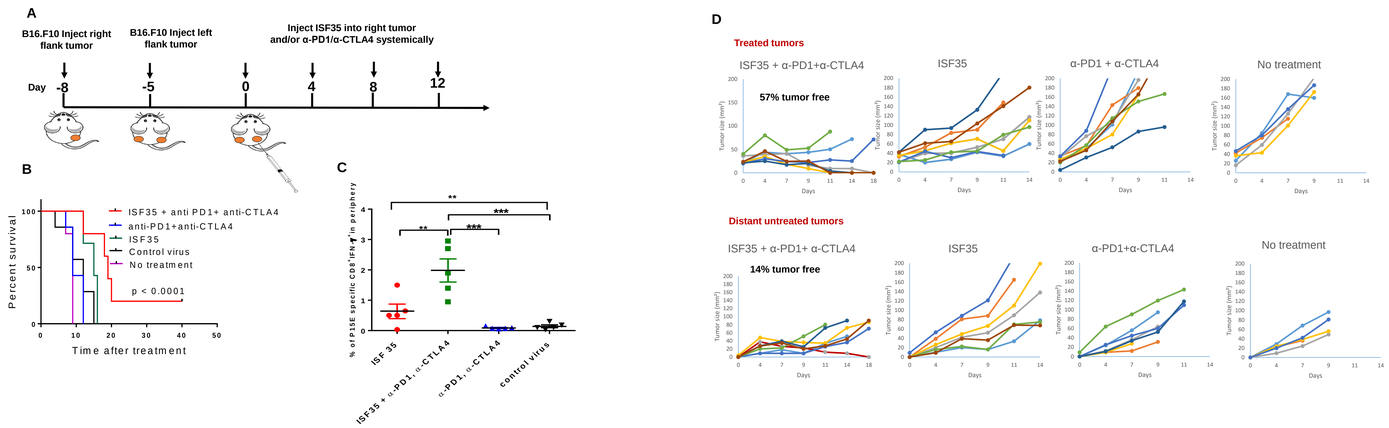


Fig.4. ISF35 and checkpoint blockades (anti-CTLA-4 plus anti-PD-1) synergize to reject local and distant tumors and generate systemic immunity: (A) treatment strategy (B) mice survival (C) tumor antigen (p15E) specific CD8 T cells in circulation and (D) growth of treated and distant B16.F10 tumors. Data 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