Inducible T cell Co-stimulator (ICOS) is upregulated on lymphocytes following radiation of tumors and ICOS agonism in combination with radiation results in enhanced tumor control.

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Abstract

Background: Radiation and co-stimulatory ligands or checkpoint inhibitors have demonstrated improved anti-tumor immunity and overall survival in preclinical animal studies. However, the results of human trials suggest we have not yet found the optimal combination. Here we demonstrate upregulation of ICOS expression on T cells following focal tumor radiation and test the hypothesis that ICOS agonism in combination with radiation will enhance the immunologic effect of radiation resulting in increased survival.
Methods: BALB/c mice bearing CT26 tumors or C57BL/6 mice bearing Panc02 tumors were treated at d14 with 20Gy CT guided radiation therapy, and anti-ICOS antibody or isotype control antibody was administered i.p. Mice were followed for overall survival to 100 days post implantation. Animals were euthanized when tumors reached 1.2cm in greatest diameter. Flow cytometry was performed using a T cell panel on fresh whole blood, PBMC, or tumor infiltrating immune cells.
Results: 24 hours following 20Gy focal radiation to a CT26 tumor there was a significant increase in the percent of circulating CD4 Treg that express ICOS in the blood (27.42% vs 18.02%, p<0.0001, n=5/group). Similarly, 7 days following radiation there was an increase in non-Treg CD4 cells expressing ICOS in the blood (7.73% vs 3.68%, p<0.0001, n=5/group) and the tumor (62.16% vs 34.04%, p=0.004, n=5/group). ICOS expression was also increased on CD8 T cells in irradiated tumors (25.34% vs 14.02%, p=0.007). In mice bearing CT26 tumors, ICOS agonist antibody was administered prior to, concurrent with, or 7 days post radiation. Concurrent administration was associated with the most significant increase in survival (50%) when compared to isotype control (0%), ICOS agonist antibody alone (10%), or radiation plus isotype (0%). In the less immunogenic Panc02 tumor model, no survival benefit was seen with radiation and ICOS therapy. However in the same model, dual PD-1 antagonism and ICOS agonism plus radiation led to a significant increase in survival when compared to all other combinations, with an increase in median survival from 46 days to 68 days, p=0.01 compared to radiation alone and was associated with a 25% long term survival.
Conclusions: ICOS is upregulated on T cells following radiation and targeting ICOS in combination with radiation is associated with improved survival. Timing appears important as the benefit is optimal when ICOS agonism is delivered concurrent with radiation rather than preceding or 7 days post-radiation. In poorly immunogenic tumors, addition of PD-1 antagonism to the combination can lead to improved survival.

Background

- Radiation therapy of tumors is associated with modification of the local and systemic immune response
- Radiation therapy has shown synergy in preclinical models with both checkpoint inhibitors (anti-PD1, anti-CTLA-4) as well as in combination with co-stimulatory molecules (anti-OX40, and 41BB)
- ICOS is an inducible co-stimulatory molecule that is expressed on activated T cells
- ICOS is a member of the CD28/CTLA4 family
- The ICOS agonist antibody JTX-2011 and other monoclonal Abs that target ICOS are in clinical development
- The mouse surrogate of JTX-2011 used in this study has been previously shown to mediate dose dependent proliferation and activation of CD4 T effector cells and selective reduction of intratumoral T regulatory cells

Results

ICOS is upregulated on circulating CD25+ regulatory T cells 24 hours and on circulating effector T cell 7 days following radiation.

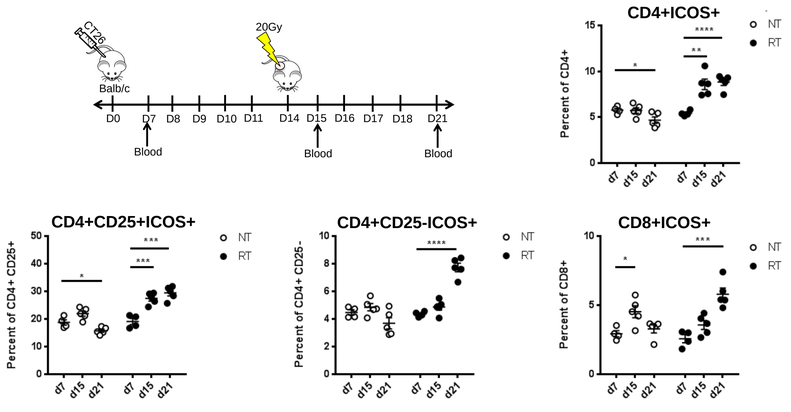

Figure 1: Balb/c mice were inoculated in the flank with 2x10^5 CT26 cells. Radiation was delivered with a single tangential beam at a dose of 20 Gy on day 14. Blood was taken on days 7, 15, and 21. Flow cytometry was performed on whole blood. NT: no treatment, RT: radiation therapy. N=5/group. * p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001

Radiation causes an increase in CD25+ T regulatory cells and in ICOS+ effector T cells in the tumor.

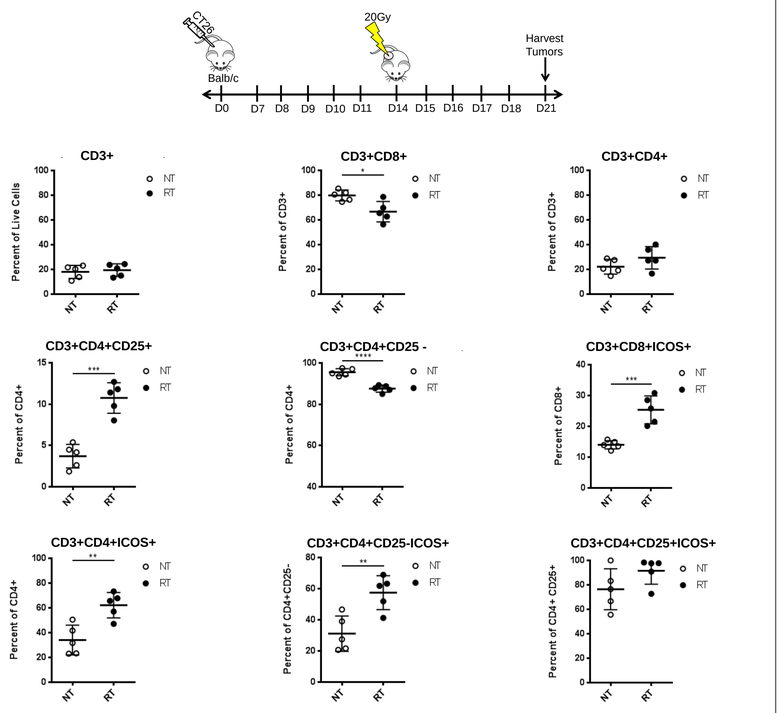

Figure 2 : Balb/c mice were inoculated in the flank with 2x10^5 CT26 cells. Radiation was delivered with a single tangential beam at a dose of 20 Gy on day 14. Tumors were harvested on day 21. Tumors were digested into single cell suspensions using a triple enzyme digest. Intratumoral cells were analyzed by flow cytometry. NT: no treatment, RT: radiation therapy. * p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001

ICOS agonist antibody treatment in combination with radiation improves survival. Concurrent administration is optimal.

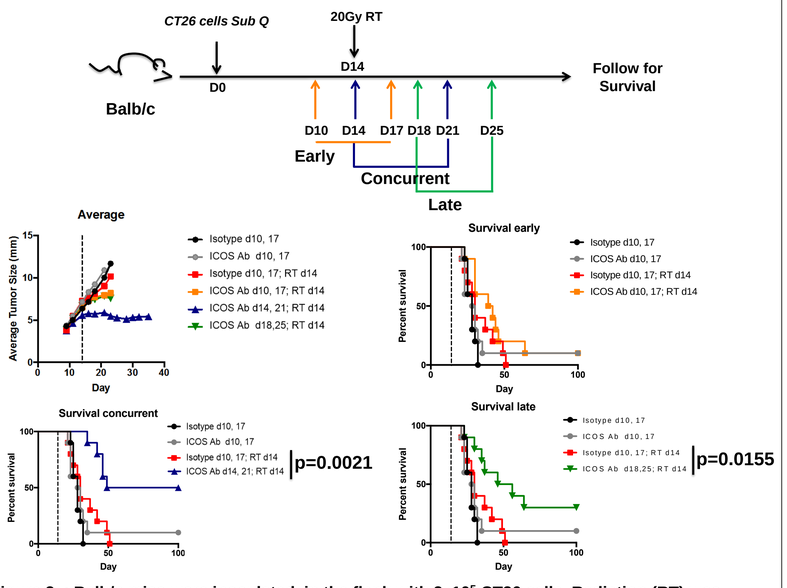

Figure 3. : Balb/c mice were inoculated in the flank with 2x10^5 CT26 cells. Radiation (RT) was delivered with a single tangential beam at a dose of 20 Gy on day 14. Isotype and anti-ICOS antibodies were administered at a dose of 0.25 mg/kg i.p. Timing of RT and antibody administration is illustrated in the diagram. N=10/group. Representative of 2 independent experiments.

ICOS agonist antibody in combination with radiation and PD-1 antagonism improves survival in poorly immunogenic tumors.

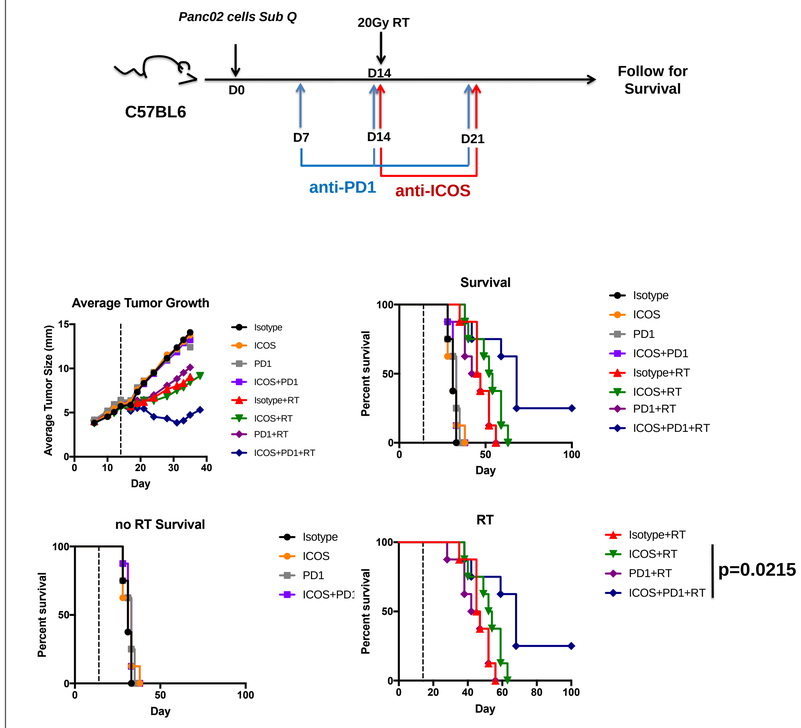

Figure 4.C57BL/6 mice were inoculated in the with a dose of 2x10^5 Panc02 cells. Radiation (RT) was delivered through a single tangential beam at a dose of 20Gy on day 14. Anti-ICOS Ab and Isotype Ab were administered i.p. at a dose of 0.25 mg/kg on days 14 and 21. Anti-PD1 Ab was administered i.p. at a dose of 250 ug/mouse on days 7,14 and 21. N=10/group. Representative of 2 independent experiments.

Conclusions

- Through analysis of the phenotype of key T cells following radiation therapy we have identified ICOS as a novel target to modulate the immune response to radiation therapy.
- There was an increase in survival associated with combined radiation treatment and ICOS agonist antibody administration, but only when treating with the ICOS antibody either concurrently with or after radiation.
- In a less immunogenic tumor model the combination of PD-1 antagonist and ICOS agonist with radiation resulted in an increase in survival relative to either agent alone with radiation. Therefore, ICOS agonism may represent a new modality for combination immunotherapy of anti-PD-1 resistant tumors.
- The therapeutic efficacy of the ICOS agonist Ab appears to require RT-mediated modulation of the tumor immune infiltrate and induction of ICOS+ CD4 and CD8 T cells.
  - The percentage of ICOS+ circulating CD4 T regulatory cells is increased within 24 hours of focal tumor radiation.
  - The percentage of ICOS+ circulating CD4 and CD8 T effector cells is increased 7 days following focal tumor radiation.
  - The percentage of ICOS+ intratumoral CD4 and CD8 T effector cells is increased 7 days following focal tumor radiation.
  - Although ICOS expression levels were not increased on these cells, focal tumor radiation also resulted in an increased percentage of intratumoral T regulatory cells.