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.

Abstract

Background: Radiation and co-stimulatory ligands or costimulatory inhibitors have demonstrated improved antitumor 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 1.2 cm in greatest diameter. Flow cytometry was performed using a 7 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 expressing ICOS in the blood (27.42% vs 18.02%, p=0.001, 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 3.88%, p=0.001, 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 24 hours 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 with 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 or antagonist are administered concurrently with radiation. In poorly immunogenic tumors, addition of PD-1 antagonism to the combination can lead to improved survival.

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 2x10⁶ 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, 14, 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.

Figure 2: Balb/c mice were inoculated in the flank with 2x10⁶ 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.

Figure 3: Balb/c mice were inoculated in the flank with 2x10⁶ CT26 cells. Radiation (RT) was delivered through a single tangential beam at a dose of 20Gy on day 14. Anti-ICOS Ab and Isotype were administered i.p. at a dose of 0.25 mg/kg on days 4 and 14. Anti-PD1 Ab was administered i.p. at a dose of 250 µg/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.

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 2x10⁶ Panc02 cells. Radiation (RT) was delivered through a single tangential beam at a dose of 20Gy on day 14. Anti-ICOS Ab and Isotype 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 µg/mouse on days 7, 14 and 21. N=10/group. Representative of 2 independent experiments.