

Development of a Preclinical Model to Test Adjuvant Immunotherapy in Combination with SBRT

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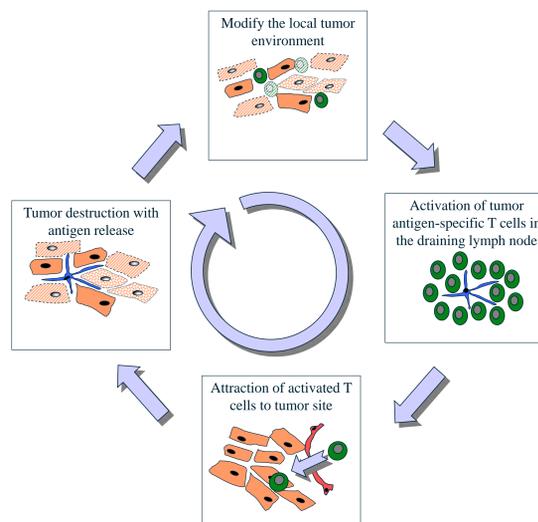
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Purpose/Objective

The purpose of this study was to characterize, in an animal model, the effect of a clinically relevant Stereotactic Body Radiation Therapy (SBRT) dose fractionation (60 Gy/3 F) on the immune system in order to develop effective adjuvant immune therapies to use in combination with SBRT for enhanced tumor control rates.

Model



Results and Methods

Focal Radiation 60 Gy in 3 fraction delays tumor growth in a s.c. 3LL lung tumor

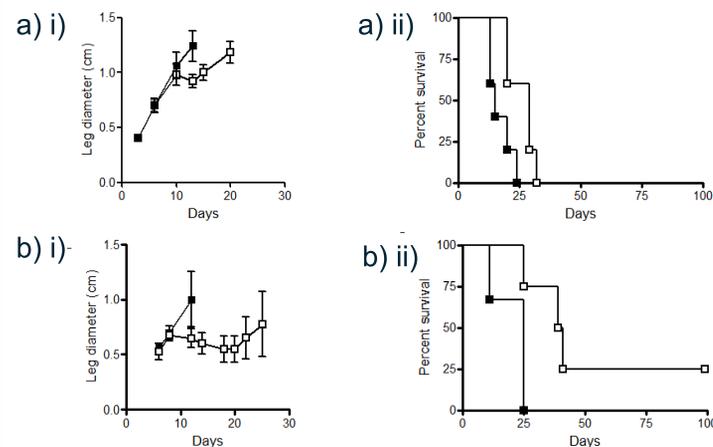


Figure 1: 3LL tumors were established s.c. in the right leg of C57BL/6 mice. Tumors were focally treated with 20Gy fractions on a) day 5 or b) day 5, 7 and 13. Mice were followed for i) tumor growth, as determined by leg diameter, and ii) survival. Graphs show untreated (filled symbols) and irradiated tumors (open symbols). Mice treated with radiation alone (60 Gy in 3 fractions) had a significant increase in median survival when compared with no radiation, 41 days versus 24.5 days respectively ($p < 0.0001$)

Changes to tumor infiltrating immune cells 48 hours post focal radiation

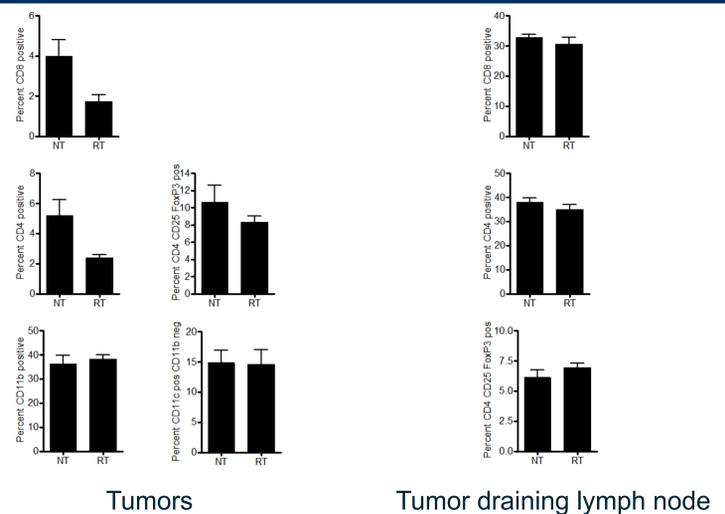


Figure 2: C57BL/6 mice were inoculated s.c. with 500,000 3LL cells into the right hind limb and 20Gy focal radiation was delivered on day 5. 48 hrs after radiation, tumors and tumor draining lymph nodes were harvested and analyzed by FACS analysis for changes in immune cells. There was a significant decrease in both CD4 and CD8 cells post radiation. Additionally a decrease in regulatory T cells was observed.

Changes to tumor infiltrating immune cells 7 days post focal radiation

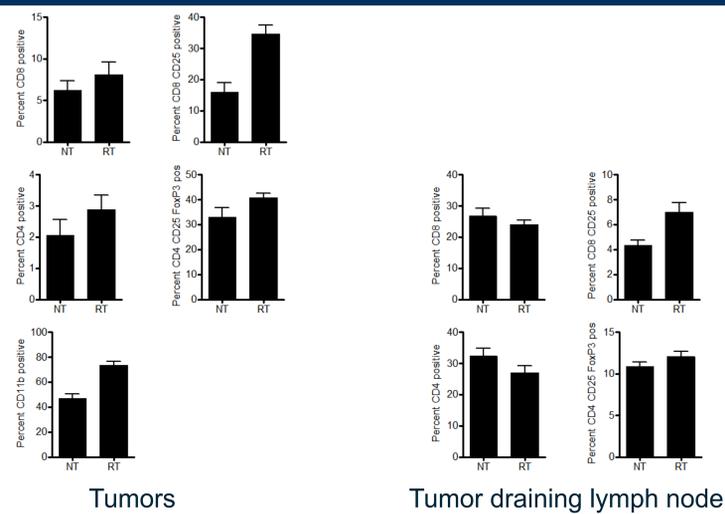


Figure 3: C57BL/6 mice were inoculated s.c. with 500,000 3LL cells into the right hind limb and 20Gy focal radiation was delivered on days 5 and 7. 7 days after the first radiation treatment, tumors and tumor draining lymph nodes were harvested and analyzed by FACS analysis for changes in immune cells. CD8 and CD4 T cell counts returned but a significantly higher fraction of CD8 T cells in the tumor had an activator effector phenotype.

CD8 T cell enhance tumor control in irradiated animals

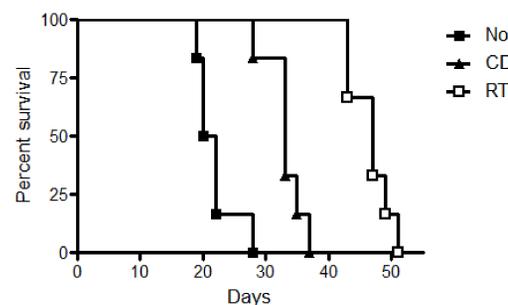
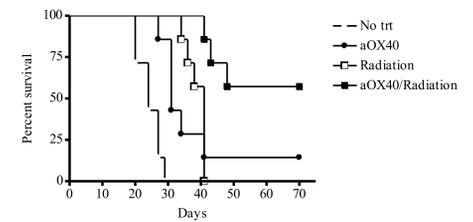


Figure 4: C57BL/6 mice were inoculated s.c. with 500,000 3LL cells into the right hind limb and 20Gy focal radiation was delivered on days 5, 7 and 13. For CD8 depletion, animals were treated i.p with 25µg Lyt 2 depleting antibody weekly. Mice that received radiation in the absence of CD8 T cells had a significant decrease in survival ($p < 0.05$) when compared to radiation in the presence of an intact immune system.

Addition of an immune adjuvant to radiation enhances survival



| Treatment Group | Naive | aOX40 | RT | RT&aOX40 |
|---------------------------|--------|----------|----------|----------|
| Percent survivors (#mice) | 0% (3) | 100% (1) | 100% (1) | 100% (3) |

Figure 5: C57BL/6 mice were inoculated s.c. with 500,000 3LL cells into the right hind limb and 20Gy focal radiation was delivered on days 5, 7 and 13. Treatment with an OX40 agonist antibody was given one day after the first radiation treatment at a dose of 250µg i.p.. Mice receiving combined therapy had a significant increase in survival when compared to either radiation or OX40 agonist antibody therapy alone ($p < 0.05$). Animals surviving were challenged on the opposite flank with 500,000 3LL cells and show long term protection against rechallenge.

Animals treated with combination therapy develop a robust cytotoxic T cell response.

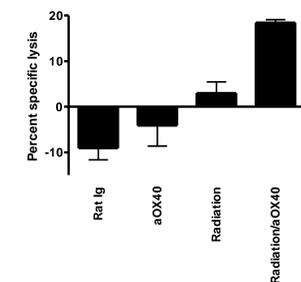


Figure 6: C57BL/6 mice were inoculated s.c. with 500,000 3LL cells into the right hind limb and 20Gy focal radiation was delivered on days 5, 7. Treatment with an OX40 agonist antibody was given one day after the first radiation treatment at a dose of 250µg i.p.. Ten days post the second radiation treatment, an *in vivo* CTL assay was performed using MUT-1 as a target antigen. Adjuvant agonist OX40 antibody therapy significantly increases the *in vivo* CTL activity when compared to radiation alone.

Conclusions

Our results suggest an intact CD8 T cell response plays a role in the local control of a mouse lung tumor treated with a clinically relevant SBRT dose fractionation. Combining this fractionation scheme with T cell co-stimulation resulted in a significant increase in survival when compared with either approach alone.

Implications:

- The immune system plays a role in the function of radiation therapy in this model
- Using a targeted immune adjuvant we can enhance radiation therapy
- These findings will be extended to patients receiving SBRT to determine the opportunity for immune therapy

Acknowledgements:

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