LOCAL RADIATION IN COMBINATION WITH CPG AND ANTI-OX40 INDUCES ENHANCED T CELL ACTIVATION AND PROLIFERATION

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Background We, and others, have previously shown that the in-situ vaccine of hypomethylated CG-enriched oligodeoxynucleotide (CpG) with agonist anti-OX40 antibody (OX40) is effective at curing mice in the A20 lymphoma model [1–4]. In separate preclinical models where CpG+OX40 fails to cause tumor regression, radiation therapy (RT) prior to the in-situ vaccine enhances the anti-tumor effect of CpG+OX40 [4]. We investigated the immune response, and specifically the activity of T cells, following treatment with RT+CpG+OX40 in the B78 melanoma model where CpG+OX40 typically fails to cause tumor regression.

Methods C57BL/6 mice were inoculated with 2x10⁶ B78 melanoma cells on the right flank and allowed to grow until the average tumor size was ~150mm³. In two independent experiments, mice were randomized (n=4–5 per group per experiment) and treated with one of the following: 1) PBS, 2) CpG+OX40, 3) RT, 4) RT+CpG+OX40. 12 Gy external beam RT was dosed to the flank tumor on day 0 and intratumoral CpG (50µg)+OX40 (20 µg) were given on days 5, 7, and 9 after RT. Spleens and tumor draining lymph nodes (TDLNs) were harvested on day 12. T cell activation and proliferation were assessed via flow cytometry.

Results Compared to all other groups in the study, mice treated with RT+CpG+OX40 demonstrated significantly elevated levels of CD4+ and CD8+ T cell activation in the TDLNs, as measured by interferon gamma expression. Similar trends of CD4+ and CD8+ T cell activation were measured in the spleens. Splenic CD8+ T cells from RT+CpG+OX40 treated mice demonstrated significantly elevated levels of proliferation over PBS and RT, as measured by Ki67.

Conclusions In B78 melanoma, a weakly immunologic tumor model, combining RT with the in-situ vaccine CpG+OX40 enhances the activity of T cells, evidenced by significantly increased CD4+ and CD8+ T cell activation in the TDLN and spleen and elevated CD8+ T cell proliferation in the spleen.

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<http://dx.doi.org/10.1136/jitc-2021-SITC2021.598>