TARGETING CCR8-EXPRESSING TUMOR INFILTRATING TREGS IN COMBINATION WITH RADIOTHERAPY ENHANCES ANTI-TUMOR IMMUNITY

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Background Tumor infiltrating Tregs (TITRs) negatively regulates anti-tumor immunity and promotes tumor progression. Increased level of TITRs has also been associated with poor prognosis in multiple cancer types which led to identification of several TITR specific therapeutic targets. Chemokine receptor CCR8 is highly enriched in the TITR population. Depletion of TITR via anti-CCR8 antibody resulted in potent anti-tumor efficacy as single agent in checkpoint inhibitor responsive syngeneic models. At Varian, immune profiling of ex vivo irradiated human head and neck tumors showed decreased viability in CD4⁺ and CD8⁺ T cells but not in the TITR population, which suggest depleting TITR as a therapeutic strategy to improve efficacy of radiotherapy (RT). Here, we hypothesized that combining anti-CCR8 with RT improves anti-tumor response via TITR depletion.

Methods To investigate the therapeutic potential of combining anti-CCR8 with RT, female Balb/c mice were orthotopically implanted with 4T1-Luc2-1A4 cells in mammary fat pad followed by focal radiation. Implanted mice were irradiated at 0 or 10 Gy on day 7 post-implantation. Anti-mouse CCR8 depleting antibody (BioLegend) or isotype control was administered intraperitoneally at 10 mg/kg on day 7, 10, and 14 post-implantation. Tumor measurements were taken three times per week for 47 days for efficacy evaluation. On day 15 post-implantation, treated and non-treated tumors were harvested for flow cytometric and bulk-RNA sequencing analyses.

Results Combining anti-CCR8 treatment with 10 Gy radiation significantly improved anti-tumor efficacy compared with anti-CCR8 treatment alone or radiation treatment alone. One day following completion of anti-CCR8 treatment, we observed increased percentage of CD8⁺ T cells (p < 0.01) and decreased percentage of TITRs (p < 0.05) in anti-CCR8-treated and irradiated tumors versus anti-CCR8 treatment alone. Tumor CD8⁺ T cells also demonstrated increased expression of activation marker CD39 (p < 0.01) in anti-CCR8 plus RT treated tumors. At the transcriptional level, combination of anti-CCR8 with radiation upregulated pathways associated with TNF-α and IFN-γ response in comparison to single-agent treatment, suggesting activation of anti-tumor immunity via enhanced T cell activation. Furthermore, anti-CCR8 plus RT significantly prolonged survival of tumor-bearing mice (78% alive) compared with anti-CCR8 treatment alone (11% alive).

Conclusions Our preclinical data demonstrate high therapeutic potential of combining anti-CCR8 depleting antibody with radiotherapy to trigger synergistic enhancement of immune response in tumors that are refractory to immune checkpoint blockade. In conclusion, targeting CCR8-expressing TITRs in combination with radiotherapy displayed superior anti-tumor activity and prolonged survival than single-agent treatment alone.

REFERENCE