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09.03 CD122-DIRECTED IL-2/ANTI-IL-2 COMPLEXES ENHANCE ABSCOPAL RESPONSES TO RADIATION COMBINED WITH ANTI-PD-1

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Background Early clinical trials have provided evidence for RT-induced systemic effects in conjunction with αPD-1 or IL-2 in metastatic patients, but strong abscopal responses are clinically rare. Dual combinations of αPD-1 with more effective and less toxic IL-2 derivatives, e.g., CD122-directed pegylated IL-2, are also currently under investigation. Whether a combination of RT, αPD-1, and CD122-directed IL-2/anti-IL-2 complexes (IL-2c) can increase abscopal effects against established non-irradiated tumors is unknown. Also, in-depth analyses of the differentiation of tumor-specific $CD8^+$ T cells have not yet been reported for αPD-1/IL-2c in combination with RT. We investigated how adding IL-2c to hRT/αPD-1 affects tumor-specific $CD8^+$ T cell differentiation in mouse tumor models and the potential of this triple combination to enhance the abscopal effect compared to the respective dual treatments.

Materials and Methods Mice bearing bilateral tumors were treated with two fractions of 8 Gy (C51 colon carcinoma model) or 12 Gy (B16 melanoma model); αPD1 was given weekly; IL-2c was given for five consecutive days. CD8 T cell-depleting and CXCR3-blocking antibodies were used to determine if the therapeutic effects depend on $CD8^+$ and $CXCR3^+$ T cells. Differentiation stages of tumor-specific $CD8^+$ T cells in tumor-draining lymph nodes, spleen, blood, and tumors were determined by flow cytometry using MHC-I tetramers and various antibodies.

Results The abscopal effect was significantly stronger in triple-treated mice compared to mice treated with RT/αPD-1 (C51 model: $P< 0.01$; B16 model: $P< 0.05$), RT/IL-2c (C51 model: $P< 0.01$; B16 model: $P< 0.001$) or αPD-1/IL-2c (C51 model: $P< 0.0001$, B16 model: $P< 0.01$). Moreover, triple therapy improved survival and resulted in complete cures of 3/12 mice in the C51 model and 2/12 mice in the B16 model. These anti-tumor effects were associated with dramatic expansion of tumor-specific $CD8^+$ T cells. Undifferentiated stem-like and effector-like but not terminally differentiated exhausted cells particularly strongly increased. Moreover, IL-2c induced CXCR3 expression on tumor-specific $CD8^+$ T cells. Both $CD8^+$ (C51 model: $P< 0.0001$; B16 model: $P< 0.01$) and $CXCR3^+$ (C51 model: $P< 0.0001$) T cells were crucial for the RT-induced abscopal effect upon RT/αPD-1/IL-2c treatment.

Conclusions RT/αPD-1/IL-2c triple treatment resulted in superior local and systemic expansion of tumor-specific $CD8^+$ T cells with stem- and effector-like phenotypes. Also, IL-2c strongly increased $CXCR3^+$ $CD8^+$ T cells that were associated with pronounced abscopal responses in models with an established metastasis resistant to αPD-1/IL-2c and only transiently responding to RT/αPD-1 or RT/IL-2c. Therefore, such triple combinations appear promising for clinical evaluation in metastatic patients.

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09.04 SECONDARY RESISTANCE TO IMMUNOTHERAPY IS ASSOCIATED WITH DEATH AND DE-DIFFERENTIATION OF ACTIVATED T CELLS

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Background Immunotherapies have transformed the care of patients with multiple tumor types, but the majority who respond eventually progress after a period of stabilization. The reasons for this are not well known. We set out to explore mechanisms of immunotherapy failure in this setting, using a murine model of melanoma treated with a regulatory T cell (Treg)-depleting antibody combined with a cancer cell vaccine.

Materials and Methods C57BL/6 mice were injected subcutaneously with B16 cells. Treatment with a mouse IgG2a depleting αCD25 antibody was given on day 5, followed by a B16