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 absorbal 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 absorbal 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 absorbal 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 absorbal 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 αPD1/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 absorbal effect upon RT/αPD1/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 absorbal 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.