our group could previously demonstrate that arming CAR T cells with C-C-motive-receptor 8 for improved tumor-directed migration along the C-C-chemokine ligand 1 - CCR8 axis and a dominant-negative receptor against TGF-β for resistance to suppression enable activity in pancreatic cancer models. The value of this approach for other entities was however unclear. We now investigated the potential of this combination for treatment of HER2-positive cancer models in conjunction with a HER2-targeted CAR.

Materials and Methods Primary murine and human T cells were isolated and activated. T cells were retrovirally transduced. Phenotype, activation, exhaustion and proliferation were monitored in vitro. Cytokine production was assessed with ELISA. In vivo, survival and tumor growth of mice that were subcutaneously injected with tumor cells and treated with CAR T cells carrying either CCR8, DNR or both receptors were measured. To look at chemokine expression in tumor material, mRNA was isolated from tumor material and RT-qPCR was performed.

Results We found that expression of CCR8 can redirect CAR T cells to the tumor and a DNR can prevent immunosuppression of CAR T cells in the tumor microenvironment. The improved functionality of CAR-CCR8-DNR T cells compared to CAR T cells against the HER2 antigen could be demonstrated in vitro and in vivo in human HER2+ tumor models. Conclusions Equipping CAR T cells with CCR8 and DNR emerges as a strategy not only limited to certain antigens, but as a potential universal approach to render cellular therapies more effective. The modularity of this concept promises further preclinical and perhaps clinical development to improve personalized immunotherapy.

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**CD122-DIRECTED IL-2/ANTI-IL-2 COMPLEXES ENHANCE ABDOPAL 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** Mouse-bearing bilateral tumors were treated with two fractions of 8 Gy (C57Bl6 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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**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