V90lec13 were evaluated with a 4-hours Calcein-AM assay or with a 40/60-hours real-time cell assay against HER-2$^+$ breast cancer cell lines. The concentration of cytokines produced upon the 20-hours co-colture of effector with target cells was assessed with a multiplex assay by flow cytometry analysis. The *in vivo* biodistribution of the fluorophore- conjugated HER2xCD3 bsAb was monitored in tumor bearing NSG mouse model.

Results HER2xCD3 binds efficiently to CIK cells with the ScFv of CD3 and to cancer cells with the ScFv of HER-2 in a dose-dependent manner. The specific combination of HER2xCD3, TRS or TRS V90lec13 with CIK cells significant enhances their anti-tumor activity against several breast cancer cell lines, compared to CIK cells alone, even at a very low effector/target ratio (0.1:1). Interestingly, TRS-resistant tumor cell lines show to be sensitive instead to HER2xCD3-redirected CIK cell lytic activity. The increase of CIK cell killing is correlated to the dose of bsAb and it is functional even at very low concentrations. Moreover, redirected-CIK cells presents a proinflammatory and not a toxic cytokines profile. The bsAb HER2xCD3 arrives efficiently at the tumor site where reaches the maximum concentration after 8 hours of injection into mice.

Conclusions These results highlight the potentiality of using clinical grade mAbs or recombinant immunotools to improve the cytotoxic activity of CIK cells against HER-2$^+$ tumor cells, opening new perspectives for adoptive immunotherapy to treat solid tumors.

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P08.02 LIPOSOMAL DOXORUBICIN ENHANCES THE RADIATION-INDUCED ABSCOPAL EFFECT BY PROMOTING THE RELEASE OF MITOCHONDRIAL DNA

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Background Localized radiotherapy (RT) can cause a T cell-mediated abscopal effect on non-irradiated tumor lesions, particularly in combination with immune checkpoint blockade (ICB). By using syngeneic tumor models, we studied whether adding low-dose doxorubicin to RT and αPD-1 can enhance the RT-induced abscopal effect.

Materials and Methods In mice bearing bilateral subcutaneous tumors, the primary tumor was irradiated with 2 × 12 Gy (B16-CD133 melanoma model) or 3 × 8 Gy (MC38 colon carcinoma model). Liposomal doxorubicin (4 mg/kg) was given i.v. once together with RT; αPD1 was given weekly. Tumor growth and survival of mice were determined (5–9 mice per group). Depleting antibodies were used to elucidate whether the abscopal effect depended on CD8$^+$ T cells. Tumor-specific CD8$^+$ T cells were determined flow cytometrically using MHC tetramers and various antibodies. Mitochondrial DNA (mtDNA) was depleted in tumor cells with Zalcitabine. *In vitro*, extracellular (e)ATP release by tumor cells was determined by CellTiter-Glo® 2.0. Tumor cell production of type I Interferon (IFNβ1) was measured by ELISA with/without incubation with cGAS-STING pathway inhibitors. CXCL10, cytosolic genomic DNA (gDNA), and cytosolic mtDNA were measured by qPCR.

Results Abscopal tumor control was as follows: RT/αPD-1/doxorubicin > doxorubicin/αPD-1 ($p < 0.01$) ≈ RT/doxorubicin ($p < 0.01$) ≈ RT/αPD-1 ($p < 0.05$) (B16 melanoma model); RT/αPD-1/doxorubicin > RT/αPD-1 ($p < 0.01$) ≈ RT/doxorubicin ($p < 0.001$) ≈ doxorubicin/αPD-1 ($p < 0.01$) (MC38 colon carcinoma model). Experiments with various inhibitors of the cGAS/STING pathway showed that liposomal doxorubicin induced type I IFN through the cGAS/STING pathway ($p < 0.05$ with vs. without inhibitors). In mtDNA-depleted tumor cells, doxorubicin induced less cytosolic mtDNA ($p < 0.001$) (but not less cytosolic genomic DNA), less IFNβ1 secretion ($p < 0.05$), less eATP release ($p < 0.0001$), and less CXCL10 ($p < 0.0001$) than in non-mtDNA-depleted tumor cells. Triple therapy with RT, αPD-1, and liposomal doxorubicin induced more mature dendritic cells ($p < 0.05$) and more tumor-specific CD8$^+$ T cells ($p < 0.01$) compared to RT/αPD-1 and doxorubicin/αPD-1 therapy. When CD8$^+$ T cells were depleted or mtDNA-depleted tumor cells were implanted, the doxorubicin-induced enhancement of the abscopal effect was abolished ($p < 0.05$).

Conclusions Single low-dose liposomal doxorubicin can substantially enhance the RT-induced abscopal effect in conjunction with αPD-1. mtDNA leakage induced by doxorubicin appears crucial for the doxorubicin-enhanced RT-induced abscopal effect. These findings may be helpful for the planning of clinical radiochemoimmunotherapy trials in (oligo) metastatic patients.

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P08.03 INTERLEUKIN-12 GENE ELECTROTRANSFER AS AN ADJUVANT IMMUNOTHERAPY TO ELECTROCHEMOTHERAPY

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