

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-culture 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-redirced 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 ABCOPAL 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 α PD-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; α PD1 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 (IFN β 1) 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/ α PD-1/doxorubicin > doxorubicin/ α PD-1 ($p < 0.01$) \approx RT/doxorubicin ($p < 0.01$) \approx RT/ α PD-1 ($p < 0.05$) (B16 melanoma model); RT/ α PD-1/doxorubicin > RT/ α PD-1 ($p < 0.01$) \approx RT/doxorubicin ($p < 0.001$) \approx doxorubicin/ α PD-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 IFN β 1 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, α PD-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/ α PD-1 and doxorubicin/ α PD-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 α PD-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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Background Electrochemotherapy (ECT) exhibits high therapeutic effectiveness in the clinic, achieving up to 80% local tumor control but without a systemic (abscopal) effect. It was proposed that ECT elicits *in situ* vaccination; therefore, we investigated its immunological effects. Moreover, we designed a combination therapy consisting of ECT via intratumoral application of bleomycin, oxaliplatin or cisplatin with peritumoral gene electrotransfer of a plasmid encoding interleukin-12 (p. t. IL-12 GET). Our hypothesis was that p. t. IL-12 GET potentiates the effect of ECT on local and systemic levels and the potentiation varies depending on tumor immune status.

Materials and Methods The combination therapy was tested in three immunologically different tumor models: B16F10 malignant melanoma, 4T1 mammary carcinoma and CT26 colon carcinoma (U34401-1/2015/7, U34401-3/2022/11). Growth of primary treated tumors and of distant untreated tumors, mimicking a systemic disease, was followed. After the therapy, cytological and histological analyses were performed to detect the types of cell death and immunologically important biomarkers as tumor immune infiltrate, the expression of MHC-1, PD-L1 and danger signals.

Results ECT induced immunogenic cell death, changes in the expression of cell markers such as MHC-1 and PD-L1 and other immunologically important danger signals. Moreover, it attracted effector immune cells intratumorally. In poorly immunogenic B16F10 melanoma, IL-12 potentiated the antitumor effect of ECT with biologically equivalent low doses of cisplatin, oxaliplatin or bleomycin. The most pronounced potentiation was observed after ECT using cisplatin, resulting in a complete response rate of 38% and an abscopal effect. Compared to B16F10 melanoma, better responsiveness to ECT was observed in more immunogenic 4T1 mammary carcinoma and CT26 colorectal carcinoma. In both models, p. t. IL-12 GET did not significantly improve the therapeutic outcome of ECT using any of the chemotherapeutic drugs.

Conclusions Electrochemotherapy induces immunologically important changes intratumorally and the effectiveness of the combination therapy depends on tumor immune status. ECT was more effective in more immunogenic tumors, but GET exhibited a greater contribution in less immunogenic tumors. Thus, the selection of the therapy, namely, either ECT alone or combination therapy with p. t. IL-12 GET, should be predominantly based on tumor immune status.

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P08.04 PHOTOTHERAPY WITH CANCER-SPECIFIC NANOPORPHYRIN POTENTIATES IMMUNOTHERAPY IN BLADDER CANCER

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Background Immune checkpoint inhibitors (ICIs) in general have shown poor efficacy in bladder cancer (BCa). The purpose of this project is to determine whether photodynamic therapy (PDT) with BCa-specific porphyrin-based PLZ4-nanoparticles (PNP) potentiated ICI.

Materials and Methods SV40 T/Ras double-transgenic mice bearing orthotopic BCa and C57BL/6 mice carrying syngeneic bladder cancer models were used to determine the efficacy and conduct molecular correlative studies.

Results PDT with PNP generated reactive oxygen species, induced protein carbonylation, and dendritic cell maturation. In SV40 T/Ras double-transgenic mice carrying orthotopic bladder cancer, the median survival was 33.7 days in the control, compared to 44.8 (p=0.0123), 52.6 (p=0.0054) and over 75 (p=0.0001) days in the anti-PD-1, PNP PDT and combination groups, respectively. At Day 75 when all mice in other groups died, only one in 7 mice in the combination group died. For the direct anti-tumor activity, compared to the control, the anti-PD-1, PNP PDT and combination groups induced a 40.25% (p=0.0003), 80.72% (p<0.0001) and 93.03% (p<0.0001) reduction, respectively. For the abscopal anti-cancer immunity, the anti-PD-1, PNP PDT and combination groups induced tumor reduction of 45.73% (p=0.0001), 54.92% (p<0.0001) and 75.96% (p<0.0001), respectively. The combination group also diminished spontaneous and induced lung metastasis. Potential of immunotherapy by PNP PDT is multifactorial.

Conclusions In addition to its potential for photodynamic diagnosis and therapy, PNP PDT can synergize immunotherapy in treating locally advanced and metastatic bladder cancer. Clinical trials are warranted to determine the efficacy and toxicity of this combination.

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P08.05 LENTIVIRAL PROTEIN VPX DELIVERY SYSTEMS AS POTENTIAL WEAPONS TO IMPROVE CYTARABINE TREATMENT RESPONSE AGAINST ACUTE MYELOID LEUKEMIA

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Background The effective treatment of the blood cancer type acute myeloid leukemia (AML) presents several challenges. One of them is resistance to Cytarabine (ara-C), which is the primary chemotherapeutic drug used as front-line treatment against AML. In 2017, it was reported that sterile alpha motif and HD-domain-containing protein 1 (SAMHD1) plays a role in ara-C resistance.¹ SAMHD1 is an enzyme that reduces the level of dNTPs in cells, thereby serving as an attractive target for AML treatment.¹ The lentiviral accessory protein Vpx, found in Simian Immunodeficiency Viruses (SIV) and Human Immunodeficiency Virus-2 (HIV-2), is known to target SAMHD1 for proteasomal degradation.² Hence, we aim to use Vpx to reduce SAMHD1 levels in AML cells to improve ara-C sensitivity.