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.


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 ani-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 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.
Methods In order to manipulate SAMDH1 levels using Vpx, different Vpx delivery systems were developed. These were virus-like particles (VLPs) packaged with different homologs of Vpx from SIV and HIV-2, and cell-penetrating peptides (CPPs) bound to either a 67 amino acid truncated SIVmac Vpx (67aaVpx) or to the WT full-length form. Two different CPPs were used in the synthesis: TAT and CPP44, the latter is based on a study by Kondo et al.3

Results Upon treating different AML cell lines with the VLPs, we observed different SAMHD1-degradation capacities of the different Vpx homologs. Vpx from SIV isolated from macaques (mac239 and mac251) performed the best, compared to Vpx from other lineages. They also increased the ara-C sensitivity of THP-1 cells, which is an AML cell line with high SAMHD1 expression levels, up to 45-fold. Vpx from HIV-2 7312a only partially increased ara-C sensitivity, while HIV-2 Rod9 Vpx did not show any SAMHD1 degradation or improvement in ara-C sensitivity despite its high packaging efficiency in the VLPs.

As for the CPPs, CPP44 bound to 67aaVpx showed better uptake and SAMHD1 degradation compared to the TAT bound 67aaVpx in THP-1 cells. Upon co-treatment with ara-C, up to a 5-fold reduction in IC50 was observed when treated with CPP44-bound 67aaVpx. In an attempt to increase efficiency, full-length Vpx-bound CPPs will be prepared, and trials using these CPPs are currently underway.

Conclusion We demonstrate that inducing SAMHD1 degradation by Vpx delivered via VLPs or CPPs efficiently improved ara-C sensitivity in AML cell lines. Since the VLPs presented a better efficiency compared to the CPPs, we are currently testing their efficiency in primary AML blasts, ex vivo. Ultimately, combining a Vpx delivery system with treatments containing ara-C could improve treatment outcomes in high-SAMHD1 patients who fail to respond effectively to ara-C treatment.

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