1. Video - TNFα sensitizes for cisplatin mediated killing allowing synergistic apoptosis of tumor cells. [Video (526KB)](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_video_2743028_nwzw19.mp4) [Source File (MP4) 526KB](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_video_2743028_nwzw19.mp4)
TC-1 cells were *in vitro* cultured and left untreated.Cell death was followed for 22 hours using Annexin-V staining and automated imaging on a BD pathway 855 imager. Every hour a picture was taken.
2. Video - TNFα sensitizes for cisplatin mediated killing allowing synergistic induction of apoptosis of tumor cells. [Video (419KB)](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_video_2743029_n5z519.mp4) [Source File (MP4) 419KB](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_video_2743029_n5z519.mp4)
TC-1 cells were *in vitro* cultured and treated with cisplatin (2 μg/ml). Cell death was followed for 22 hours using Annexin-V staining and automated imaging on a BD pathway 855 imager. Every hour a picture was taken.
3. Video - TNFα sensitizes for cisplatin mediated killing allowing synergistic induction of apoptosis of tumor cells. [Video (655KB)](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_video_2743030_nmzm19.mp4) [Source File (MP4) 655KB](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_video_2743030_nmzm19.mp4)
TC-1 cells were *in vitro* cultured wer treated with TNFα (250iU). Cell death was followed for 22 hours using Annexin-V staining and automated imaging on a BD pathway 855 imager. Every hour a picture was taken.
4. Video - TNFα sensitizes for cisplatin mediated killing allowing synergistic induction of apoptosis of tumor cells. [Video (561KB)](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_video_2743031_ntzt19.mp4) [Source File (MP4) 561KB](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_video_2743031_ntzt19.mp4)
TC-1 cells were *in vitro* cultured and treated with cisplatin (2 μg/ml) and TNFα (250iU). Cell death was followed for 22 hours using Annexin-V staining and automated imaging on a BD pathway 855 imager. Every hour a picture was taken.
5. Supplemental figure 1) Peptide vaccination synergizes with cisplatin, which allows lowering of the cisplatin dose. [PDF (113KB)](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_supp_2743024_nhzh18.pdf) [Source File (EPS) 3634KB](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_supp_2743024_nhzh18.eps)
Wild-type C57BL/6 mice were injected with 1x105 TC-1 tumor cells. Eight days later, when tumors were palpable, mice were treated systemically with chemotherapeutics with or without addition of HPV16 E743-77 peptide in Montanide in the opposing flank. Chemotherapy was repeated one week after initial treatment and vaccination 2 weeks after initial treatment. Tumor growth was measured twice weekly. A) Numbers indicate how many mice are still alive on day 63 (or day 50 for carboplatin + peptide). Shown is the TC-1 tumor outgrowth measured two-dimensionally (mm2) after tumor challenge. Each line represents a single mouse, and eight mice per group were used. Shown is data from one representative experiment. Experiments with doxorubicin and oxaliplatin were performed once. All other combinations are representative of two or more experiments. B) Body weight for mice treated with 4 (low dose) and 10 mg/kg (high dose) cisplatin. C) Wild-type C57BL/6 were injected with C3 tumor cells. Two weeks later, when tumors were palpable, mice were treated systemically with chemotherapeutics with or without addition of HPV16 E743-77 peptide in Montanide as above. Chemotherapy was repeated one week after initial treatment and vaccination was repeated 2 weeks after initial treatment. Data represents tumor growth in each mouse. Shown are pooled data from 2 individual experiments, each with six mice per group. D) Wild-type C57BL/6 were injected with TC-1 tumor cells. One day before treatment mice were injected with anti-CD8 antibody or anti-CD4 antibody. Kaplan-Meier survival plot (\*P<0.05 and \*\*P<0.01).
6. Supplemental figure 2) Activation markers on T cells are not affected by cisplatin treatment. [PDF (39KB)](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_supp_2743025_nvzv18.pdf) [Source File (EPS) 886KB](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_supp_2743025_nvzv18.eps)
Six mice were injected with 1 x 105 TC-1 tumor cells on day 0. On day 15, mice were treated with 4 mg/kg cisplatin, i.p. Two days later mice were sacrificed and CD8 and CD4 T cells from spleens, lymph nodes and blood were stained for multiple activation markers. Data shown are from tumor draining lymph nodes (tdLN). Similar results were observed in spleens, non-tumor draining LNs, and blood. Shown is representative data of two individual experiments with 6-8 mice per group.
7. Supplemental figure 3) Synergistic effects between cisplatin and peptide vaccination preserved with other treatment protocol [PDF (31KB)](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_supp_2743026_nnzn18.pdf) [Source File (EPS) 906KB](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_supp_2743026_nnzn18.eps)
Mice were inoculated on day 0 with 1 x 105 tumor cells. On day 9, when a tumor of 4-10 mm2 was present, mice were treated with HPV16 E743-77 peptide. Six days later, on day 15, half of these mice received cisplatin treatment. Shown is the TC-1 tumor outgrowth measured two-dimensionally (mm2) after tumor challenge. Each line represents a single mouse. Six mice were left untreated, seven mice were treated with cisplatin, eight mice with peptide and eight mice with peptide and cisplatin (4 mg/kg). Numbers indicate the number of mice alive on day 57 after tumor challenge.
8. Supplemental figure 4. TNFα sensitization for chemotherapy is dose dependent. [PDF (75KB)](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_supp_2743027_n9zgc6.pdf) [Source File (EPS) 1592KB](http://ccr.msubmit.net/ccr_files9b/2014/11/13/00136461/01/136461_1_supp_2743027_n9zgc6.eps)
A) Mice were inoculated on day 0 with 1 x 105 tumor cells. On day 9, when a tumor of 4-10 mm2 was present, mice were treated with HPV16 E743-77 peptide. Six days later, on day 15, half of these mice received cisplatin treatment. Tumors were dissected on day 17, stained with TUNEL technique to detect apoptotic cells and analysed while blinded for treatment. B) TC-1 cells were in vitro exposed to cisplatin and indicated dosages of TNFα. Dose response of TC-1 cell survival upon exposure to TNFα in the presence of 2 µg/ml cisplatin as measured by MTT assay. Representative result of 2 independent experiments. C) TC-1 cells were incubated overnight after which cisplatin and/or TNFα or supernatant of overnight cultures of peptide treated tumor cells was added for an additional 22 hours. Cell death was analysed by flow cytometry analysis using Annexin-V staining. D) Dose response of MCA205 tumour cell survival upon exposure to cisplatin in the presence (various intensities of blue, amount indicated in figure) or absence (light blue) of TNFα as measured by MTT assay. E) Various concentrations of supernatant of PHA stimulated PBMCs (percentages are indicated in figure) were added to HeLa tumor cells and coincubated with 2 µg/ml cisplatin. 30 hours later cell death was measured by MTT assay. F) TC-1 tumor cells were *in vitro* exposed to indicated concentrations of topotecan, paclitaxel or carboplatin. Cell death was measured by Annexin-V staining and automated imaging on a BD pathway 855 imager. Representative for 3 independent experiments.