Background Approved anti-PD-1/PD-L1 immunotherapy has demonstrated clinical benefits for breast cancer (BC) mostly in triple-negative breast cancer (TNBC) patients. Developing effective immunotherapies for breast cancer is slow in part due to the lack of appropriate models to evaluate novel agents. In this study, we evaluated efficacy, safety, and effect on survival of neoadjuvant in situ intratumoral immunotherapy with noninfectious empty cowpea mosaic virus (eCPMV) nanoparticles in spontaneous canine mammary cancer (CMC) patients. CMC shares clinical, pathologic, biologic, and immune similarities to human BC.

Methods Eighteen companion CMC patients were enrolled in the study approved by the Ethics Committee (Study #04/2018). Dogs with poor prognosis received adjuvant medical therapy (oral metronomic cyclophosphamide and firocoxib). Seven dogs received surgery, and eleven dogs received neoadjuvant in situ intratumoral eCPMV immunotherapy (0.2 mg per injection in the target tumor) at day 0 (D0) and at D6-D9, surgery at D12-D17, and adjuvant medical therapy. Efficacy was evaluated by tumor growth inhibition (TGI), safety by hematologic, and biochemistry changes in blood, and patient outcome by survival analysis. eCPMV-induced changes in blood cells were analyzed by flow cytometry.

Results Two neoadjuvant in situ intratumoral eCPMV injections generated tumor reduction of injected tumors in all patients by surgery day without systemic adverse events. TGI ranged from 6% to 63%. Remarkably, TGI was also observed in non-injected lesions (abscopal effect) both in the ipsilateral (TGI=1% to 96%) and contralateral (TGI=10% to 78%) mammary chains. Efficacy was independent of tumor size, clinical stage, histopathologic grade, and tumor receptor status (eight cases were luminal A, one luminal B, and two TN). Values stayed within normal range for hematocrit and hemoglobin and biochemistry (total proteins, glucose, creatine, alanine aminotransferase, and urea), confirming that eCPMV immunotherapy is not toxic. eCPMV injections induced a significant increase in the number of mature and immature blood neutrophils at D6-D9 (p<0.05 for both relative to D0). Flow cytometry of circulating immune cells identified minor fluctuations induced by eCPMV injections in various immune cell subsets (not significant). eCPMV-treated patients had a statistically significant (p=0.006) improved overall survival compared to patients not treated with eCPMV.

Conclusions Neoadjuvant in situ intratumoral eCPMV immunotherapy demonstrated anti-tumor efficacy and improved survival in CMC patients without systemic adverse effects. This novel immunotherapy could be a groundbreaking immunotherapy for CMC patients and a potential future immunotherapy for human BC patients beyond the current TNBC subtype.

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