Supplementary Data

Canine patient recruitment and selection criteria

Given the rarity of IMC and the scarcity of specific beneficial therapies [1,2], any female canine patients diagnosed with IMC were recruited. Ten IMC patients were enrolled in this study (Table 1). Two clinical presentations of IMC have been recognized: primary IMC spontaneously occurs in dogs without any previous mammary nodules; secondary IMC arises in patients with a history of mammary tumors that develop inflammatory signs [1]. One dog was diagnosed with a primary IMC and nine dogs were diagnosed with secondary IMC (Table 1). Inclusion criteria at diagnosis included female dogs without evidence of metastatic dissemination, no severe infection of the target tumor, no chronic-life threatening disease or any systemic disease that could influence the immune system response (such as endocrinopathies, immune-mediated disease, leishmaniasis and ehrlichiosis), and no treatment with immunosuppressive drugs. The histopathological classification of tumors was performed according to the veterinary histological classification [3], and the histological grade of malignancy was performed as previously described [4].

Collection of tumor biopsies and surgical procedures

For the collection of the incisional tumor biopsy, all patients were sedated (Medetomidine, methadone (10 and 300 micrograms/kg, intramuscularly, respectively)) when the incisional biopsy was collected and, when necessary, analgesia was provided (Tramadol, 2 mg/kg, orally, every 12 h for 4 days). If dogs were not under sedation, the intratumoral eCPMV inoculation was performed under topical local anesthesia using a tetracaine ointment.

For surgical procedures, all patients were premedicated with medetomidine and methadone (10 and 300 micrograms/kg, intramuscularly, respectively), followed by induction with propofol (1mg/kg, intravenously) and inhalational anesthesia with isoflurane (1.5%-2.5%). Intravenous

cephazolin was given 20 min before surgery (22mg/kg). Further, depending on the mastectomy procedure, transversus abdominis plane block with bupivacaine (up to 2 mg/kg) and/or epidural anesthesia using morphine 0.1 mg/kg plus bupivacaine (up to 2 mg/kg) was provided. Diffusion catheters were placed during surgery (DC Mila International Inc®) in order to administer bupivacaine (1-2 mg/kg every 6h) in the post-operative period. Soft sterile wound dressings and a tubular mesh were placed to cover the wound. Post-surgical therapy also included firocoxib (5 mg/kg, orally every 12 h for 7 days) and tramadol (3mg/kg, orally every 12 h for 3 days). No post-operative antibiotics were prescribed. Catheters were left in place for 3 days. Wounds healed uneventfully and skin sutures were removed after 12 days.

eCPMV nanoparticles and dosage

CPMV particles devoid of RNA1 or RNA2 (empty CPMV or eCPMV) were produced through agroinfiltration of *Nicotiana benthamiana* plants using vector pEAQexpress-VP60-24K [5]. For eCPMV purification, infiltrated leaves were harvested 7 days post infiltration and homogenized with 2-3 vol of 0.1 M potassium phosphate (KP) buffer (pH 7.0) followed by addition of ¼ vol of a 20% PEG solution in water with 1 M NaCl to the clarified homogenate; PEG precipitation was carried out by stirring overnight at 4ºC. After 2x centrifugation (27,000g, 15 mins, at 4ºC) the pellet was resuspended in 0.01 M KP buffer and ultracentrifuged (130,000g, 3 hours, at 4ºC) over a 30% sucrose cushion. The sucrose fraction was collected, dialyzed against 0.1 M KP buffer, and characterized using size exclusion chromatography, native and denaturing gel electrophoresis, ultraviolet-visible spectroscopy, and transmission electron microscopy (Figure S8).

eCPMV doses were based on a previously published study in canine oral melanoma patients [6]. Briefly, the eCPMV nanoparticles were diluted in 0.5 ml of sterile phosphate buffered saline (PBS) and injected using a 25G needle (Fig. S1). The injected PBS volume was equally distributed in 3

to 5 locations within a treated tumor. The total volume of PBS was equally distributed when applying injections. The amount of eCPMV per treatment was constant regardless of the tumor size.

Medical therapy

The medical therapy consists of a cyclooxygenase-2 (COX-2) inhibitor (firocoxib, 5mg/kg; daily; oral), cyclophosphamide-based metronomic chemotherapy (12.5 mg/m^2; daily; oral) and toceranib phosphate (at 2.4-2.7 mg/kg/oral 3 days per week)[7].

Histopathological assesment of tumor necrosis area

Hematoxylin and eosin (H&E)-stained tumor tissue sections were used for histopathological diagnosis and the assesment of tumor necrosis area. Tumor necrosis area was evaluated by CellSense Entry software (Olympus, Waltham, MA, USA) measuring areas of neutrophil-associated necrosis and non-neutrophil-associated necrosis on whole microscopic slides, and calculating the percentage of necrosis as necrosis area/total tumor area*100. Total necrosis was defined as the summatory of neutrophil-associated necrosis and non-neutrophil-associated necrosis.

Immunohistochemistry (IHC) assays

Tumor biopsies from P1, P2, and P5 (pre- and post-treatment) and three control dogs were available for IHC. Unfortunately, the pre-treatment biopsy from P1 consists mostly of skin tissue with a few tumor cells and immune cells (~5% and ~20% of tumor and immune cells, respectively); P2 post-treatment biopsy was taken at necropsy 24 h after the dog died, affecting the quality of collected tumor samples. Histopathologic analysis demonstrated good tissue quality in the necropsy tissue. P3 and P4 patients were referred to our Hospital with documented IMC diagnosis,

undergone a pre-treatment biopsy (not available to us), and they died outside our Hospital without tumor biopsy collection. Single 3 µm tumor tissue cuts were used for the immunostaining of Ki-67, Cleaved Caspase-3 (CC3), myeloperoxidase (MPO), and interleukin-8 (IL-8). Deparaffination and antigen retrieval were performed in a PT Lab Vision module (Thermo Fisher Scientific Inc, Waltham, MA, USA) by immersion in 1mM EDTA buffered solution at 95 °C for 20 minutes, the sections were cooled down, and immunolabelled in an automatic autostainer (Autostainer 480S, Thermo Fisher), using a polymer-based method and a peroxidase detection system (UltraVision Quanto MAD-021881QK, Master diagnostic, Granada, Spain). Antibodies against Ki-67 (Master Diagnóstica, Sevilla, Spain; Cat. No. MAD-000310QD ready-to-use; clone SP6), CC3 (Cell Signaling, Danvers, Ma, USA; Cat. No. 9661L; 4µg/ml), MPO (Dako, Santa Clara, Ca, USA; Cat.No. A0398; 33 µg/ml), IL-8 (Abcam, Cambridge, UK; Cat. No. Ab106350; 2µg/ml), CD3 (Dako, Santa Clara, Ca, USA; Cat.No. A045201; 6µg/ml), CD20 (Thermo fisher scientific, Walham, Ma, USA; Cat. No. RB-9013; 0.33 µg/ml), FoxP3 (Master Diagnóstica, Sevilla, Spain; Cat. No. MAD-000536QD ready-to-use; clone SP97) were used as recommended by manufacturers. External controls (canine thymus for CC-3 and FoxP3; neutrophilic sebaceous adenitis for MPO and IL-8; canine lymph node for CD3 and CD20) and internal controls (tumor mitotic figures for Ki-67) were used. The corresponding negative control slides were obtained by replacing the primary antibody with a nonreacting antibody on canine tissues.

Scoring of IHC markers.

Proliferation and apoptosis indexes were defined as the percentage of positive tumor cells with the Ki-67 and CC3 markers, respectively, by counting positive and negative nuclei in 10 high-power-fields (40×). MPO was quantitatively evaluated with the binary image thresholding method as the percentage of positive area in ten 100x fields. IL-8 was semi-quantitatively scored as negative (0),

low (1+), moderate 2+ and strong (3+) extracellular stromal immunolabeling. The number of B ($CD20^+$), T ($CD3^+$), and T regulatory lymphocytes ($FoxP3^+$) was assessed in hot spots (lymphocytic-rich areas) as the number of positive cells/mm^2. T regulatory lymphocytes/T lymphocyte ratio was calculated dividing the number of $FoxP3^+$ cells/mm^2 by the number of $CD3^+$ cells/mm^2.

Cytokine measurement

The MILLIPLEX® Canine Cytokine/Chemokine Magnetic Bead Panel was used to measure 13 cytokines in plasma samples as indicted by the manufacturer (Merck Millipore, Burlington, MA, USA): GM-CSF, IFN-γ, KC (CXCL1), IP-10 (CXCL10), IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, MCP-1 (CCL2), and TNF-α.

Statistical analyses

Primary outcomes were efficacy, measured by reduction in tumor volume, biosafety by evaluation of hematological and biochemistry changes in blood and plasma, and survival of treated patients by tracking physical status of patients. For evaluation of individual eCPMV-induced changes in tumor size between start of treatment and after the first and second eCPMV injections, linear regression analysis of percentage of changes in tumor volumes by days for each tumor was performed. To evaluate potential toxic and immunological effects of eCPMV therapy in dogs, a two-tailed Student's t-test or Wilcoxon test were performed as appropriate to compare eCPMV-induced changes in blood cell numbers and plasma levels of total proteins (albumin and globulins), glucose, urea, creatinine, and ALT, and cytokine levels in samples collected before and during treatment, and at surgery. Individual changes in blood parameters and IL-8 levels were analyzed by linear regression analysis. Survival was calculated from the date of diagnosis with death from mammary cancer scored as an event and censoring of other patients at the date of last follow-up

or non-disease-related death. The Kaplan-Meier method with the log-rank test was used to estimate survival. The presence of necrosis and the immunolabeling of the IHC markers were compared between the pre-treatment/control and post-treatment biopsies to determine the effect of the eCPMV on the tumor tissue. Unpaired Student t-test or Mann-Whitney U test was used to compare continuous variables between groups. The Fisher's exact test and the Chi-square test were used to compare binary categorical variables and categorical variables with more than two categories, respectively. Pearson's correlation coefficient was used to evaluate the correlation between continuous variables. Two-tailed P values less than 0.05 were considered statistically significant. Statistical analyses were carried out using IBM SPSS Statistics program (version v.25; Armonk, NY, USA) and GraphPad Prism (version 7.02; GraphPad San Diego, CA, USA) software.

Supplementary figures and tables.

Figure S1. *In situ* eCPMV injections. The eCPMV nanoparticles were diluted in 0.5 ml of sterile phosphate buffered saline (PBS) and injected using an insulin syringe (25G needle without dead volume). The injected PBS volume was equally distributed in 3 to 5 locations within the injected tumor to perfuse the tumor with the nanoparticles as much as possible. For every eCPMV injection, the needle was inserted as illustrated above and the nanoparticles were slowly injected while the needle was slowly retracted. Pressure was put on the injection site before taking the needle out to avoid leakage.

Figure S2. Neoadjuvant *in situ* eCPMV immunotherapy does not affect red blood cell and hemoglobin levels in treated canine IMC patients. Changes induced by eCPMV injections in hematocrit (A) and hemoglobin (B). Black circles indicate basal levels at D0, blue rectangles, at D7, and brown triangles at D14. NR, refers to normal range values.

Figure S3. Neoadjuvant *in situ* eCPMV immunotherapy does not affect hepatic, renal and digestive functions in the vaccinated canine IMC patients. Changes induced by eCPMV

injections in (A) protein (albumin and globulins), (B) glucose, (C) creatinine, (D) urea, and (E) ALT levels. Black circles indicate basal levels at D0, blue rectangles, at D7, and brown triangles at D14. NR, refers to normal range values.

Figure S4. Neoadjuvant *in situ* eCPMV immunotherapy induced minimal changes in peripheral blood immune cells. Changes induced by eCPMV injections in lymphocytes (A), monocytes (B), mature neutrophils (C), and immature neutrophils (D). Black circles indicate basal levels at D0, blue rectangles, at D7, and brown triangles at D14. NR, refers to normal range values.

Figure S5. Gating strategy for immunophenotyping of canine PBMCs. (A) Leukocytes (SSC-A/FSC-A) were further defined as single (doublet exclusion FSC-H/FSC-A) live cells (SSC-A/Viability Aqua). (B) Live leukocytes were further discriminated in $CD45^+$ leukocytes and $CD14^+CD45^+$ monocytes. (C) Differential expression of MHCII and CD4 on monocytic population. (D) $CD45^+$ leukocytes were divided into lymphocytes ($CD22^+$ B cells and $CD5^+$ T cells) and (E) a CD22-CD5- cell population further characterized into MHCII+ antigen presenting cells and $CD4^+$ neutrophils ([8] and references therein). (F) Gating for $GzmB^+CD3$- NK cells. (G) Identification of $CD4^+$ T helper and $CD8^+$ T cytotoxic cells within the $CD5^+$ T cell population, and $FoxP3^+$ regulatory T cells differentially expressing CD25 within the $CD4^+$ T cell population (H). (I) Expression of cytotoxic cell marker GzmB within the $CD8^+$ T cell population. Parent population is indicated above the plots.

Figure S6. Neoadjuvant *in situ* eCPMV immunotherapy induced a differential effect on IL-8 in canine IMC patients. eCPMV injections induced an increase in IL-8 in P1 during the 14 day treatment, and a transient increase in P3, P4, and P5 by D7 with a subsequent decrease by D14. IL-8 levels in P1 were significantly higher at D14 compared to day 0 (P=0.039; linear regression analysis). Black circles indicate basal levels at D0, blue rectangles, at D7, and brown triangles at D14.

Figure S7. Neoadjuvant *in situ* eCPMV immunotherapy induces T lymphocyte infiltration and depletion of T regulatory lymphocytes in tumor samples. Representative immunostaining of tumor tissues from pre-treatment and post-treatment (P1) samples. A strong infiltration of T lymphocytes ($CD3^+$) is observed in post-treatment tumor tissue compared to pre-treatment tissues.

eCPMV treatment markedly reduces T regulatory lymphocytes (FoxP3$^+$) in post-treatment tumor tissue.

Figure S8. Transmission electron microscopy images of negatively stained eCPMV at 49,000x enlargement. White bar = 50 nm.

Table S1. Tumor changes in IMC patients by iRECIST criteria.

Table S2. List of monoclonal antibodies used for flow cytometry.

Table S3. Blood cell, biochemistry and cytokine changes during eCPMV immunotherapy in canine IMC patients.

Table S4. Blood cell and protein changes in untreated control canine IMC patients.

Table S5. Changes in the percentage of CD8, Treg and GZMB cells during eCPMV immunotherapy.

Table S6. Histopathological and Immunohistochemical changes induced in tumor samples by neoadjuvant *in situ* eCPMV therapy.

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Table S1. Tumor changes in IMC patients by iRECIST criteria.

Patient	Day	eCPMV dose, mg	Treatments	Tumor size, cm	%Change	iRECIST
eCPMV treated canine patients						
P1 (T1)	D0	0.200	8	11.0	0.0	
	D7	0.200		11.0	0.0	SD
	D19	0.200		9.4	-14.5	SD
	DFU			9.6	-13.0	SD
P1 (T2)	D0		6	4.1	0.0	
	D7			5.0	22.0	PD
	D19	0.200		5.7	39.0	PD
	DFU			9.3	127.6	PD
P2	D0	0.400	7	15.7	0.0	
(T1&T2)*	D7	0.400		15.1	-3.8	SD
	D14	0.400		13.9	-11.5	SD
	DFU	0.400		19.6	24.8	PD
P3	D0	0.400	2	19.6	0.0	
	D7	0.400		20.2	3.1	SD
	D14			20.1	2.6	SD
P4	D0	0.400	3	17.3		
	D7	0.400		16.6	-3.8	SD
	D15	0.400		17.8	3.4	SD
P5	D0	0.200	2	3.9		
	D9	0.200		3.5	-9.1	SD
	D17			2.9	-24.9	SD
Control canine IMC patients						
P6	D0			14.0		
	D25			15.9	13.6	SD
P7	D0			7.0		
	NA			NA		
P8	D0			4.0		
	D73			4.8	26.3	PD
P9	D0			5.0		
	D35			7.0	40.0	PD
P10	D0			3.0		
	D39			4.5	50.0	PD

Legends: D0, D7, D14, DFU, day 0, 7, 14, and at last follow-up (D92 in P1 and D79 in P2), respectively; T1, target tumor; T2, second tumor treated; SD, stable disease; PD, progressive disease. *, the eCPMV doses in P2 are shown as the sum for the two tumors.

Table S2. List of monoclonal antibodies used for flow cytometry.

Antibody	Clone	Fluorochrome	Provider, cat. #
CD45	YKIX716.13	eFluor 450	eBioscience™, ThermoFisher 48-5450-41
CD25	P4A10	Super Bright 600	eBioscience™, ThermoFisher, 63-0250-42
CD4	YKIX302.9	Super Bright 645	eBioscience™, ThermoFisher, 64-5040-42
CD8a	YCATE55.9	Super Bright 702	eBioscience™, ThermoFisher, 67-5080-42
EOMES	WD1928	PerCP-eFluor 710	eBioscience™, ThermoFisher, 46-48877-42
CD22	RFB-4	PE	ThermoFisher, MHCD2204
FOXP3	FJK-16s	PE-Cyanine7	eBioscience™, ThermoFisher, 25-5773-80
MHC Class II	YKIX334.2	APC	ThermoFisher, 17-5909-42
Ki-67	SolA15	Alexa Fluor 700	eBioscience™, ThermoFisher, 56-5698-82
CD5	YKIX322.3	APC-eFluor 780	eBioscience™, ThermoFisher, 47-5050-42
CD14	M5E2	Brilliant Violet 785	Biolegend, 301840
CD3	CA17.2A12	FITC	BioRad, MCA1774F
Granzyme B	Granzyme B	PE-CF594	BD, 562462
Dead cells		Zombie Aqua™ Fixable Viability Kit	Biolegend, 423102

Table S3. Blood cell, biochemistry and cytokine changes during eCPMV immunotherapy in canine IMC patients.

Variable	Day 0	Day 7	Day 14	P value*
Total leukocytes (cells/μL)	12452.0 ± 1303.0	9242.0 ± 1414.1	13920.0 ± 3417.4	**0.043**; 0.625; 0.080
Total lymphocytes (cells/μL)	1144.6 ± 334.7	925.4 ± 217.5	1438.4 ± 298.9	0.576; 0.536; 0.097
Total monocytes (cells/μL)	1073.6 ± 85.3	896.8 ± 172.6	747.0 ± 253.1	0.202; 0.242; 0.382
Total mature neutrophils (cells/μL)	9902.8 ± 1144.6	6894.8 ± 1021.2	11099.4 ± 2979.8	**0.043**; 0.686; 0.080
Total immature neutrophils (cells/μL)	0.0	84.8 ± 29.2	448.4 ± 212.3	0.068; **0.043**; 0.166
Total proteins (g/dL)	7.4 ± 0.4	7.3 ± 0.4	6.4 ± 0.4	0.655; 0.273; 0.655
Interleukin-8 (pg/mL)	1366.5 ± 798.9	4296.9 ± 1055.9	2036.8 ± 1496.2	0.068; 0.593; 0.285

Legends: ± denotes standard error; *, P values estimated by Student-T test or Wilcoxon test for day 7 and day 14 compared to day 0, and between day 7 and 14, respectively.

Table S4. Blood cell and protein changes in untreated control canine IMC patients.

Variable	Day 0	Day 14	P value*
Total leukocytes (cells/μL)	7860.0 ± 599.7	7333.3 ± 1975.1	0.593
Total lymphocytes (cells/μL)	1530.0 ± 387.7	861.3 ± 214.8	0.109
Total monocytes (cells/μL)	292.4 ± 78.4	658.7 ± 513.1	0.593
Total mature neutrophils (cells/μL)	5664.8 ± 241.2	5555.3 ± 1341.3	0.593
Total immature neutrophils (cells/μL)	40.8 ± 26.5	54.0 ± 29.1	0.593
Total proteins (g/dL)	6.4 ± 0.3	5.8 ± 1.2	0.655

Legends: ± denotes standard error; *, P values estimated by Student-T test or Wilcoxon test.

Table S5. Changes in the percentage of CD8, Treg and GZMB cells during eCPMV immunotherapy.

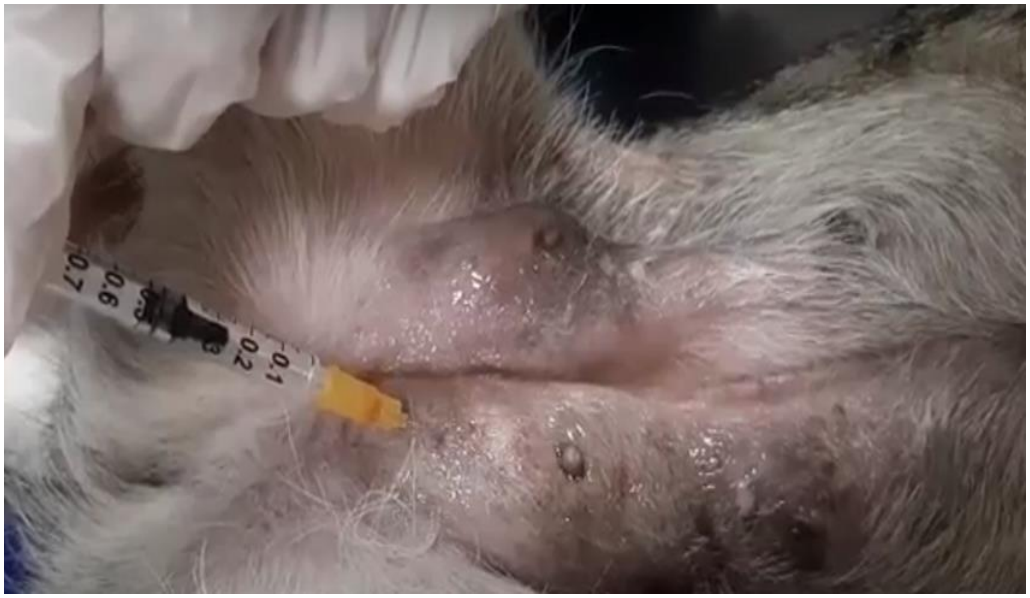
Variable	Patient	Day 0	First isolation after treatment
$CD8^+$ cells	P1	25.80	37.30
	P2	44.30	34.10
	P4	46.00	46.30
	P5	45.70	49.80
$FoxP3^+$ (Treg) cells	P1	19.40	21.30
	P2	13.50	9.39
	P4	15.10	13.30
	P5	12.00	10.70
$Treg^+/CD8^+$ cells ratio	P1	0.75	0.57
	P2	0.30	0.28
	P4	0.33	0.29
	P5	0.26	0.21
$CD8^+$ $GZMB^+$ cells	P1	28.90	30.30
	P2	77.90	59.60
	P4	22.40	14.40
	P5	69.80	63.40

Legends: GZMB: granzyme B; See supplementary Fig. S4 for gating information.

Table S6. Histopathological and Immunohistochemical changes induced in tumor samples by neoadjuvant *in situ* eCPMV therapy.

Markers	Pre-treatment n=6	Post-treatment n=3	P value*
Total necrosis (%) [a]	0.21 ± 0.31	2.85 ± 2.27	**0.024***
Neutrophil-associated necrosis (%)	0.01 ± 0.02	2.36 ± 2.49	**0.024***
Non-neutrophil-associated necrosis (%)	0.21 ± 0.31	0.49 ± 0.30	0.262*
MPO (%)	1.1 ± 0.4	9.59 ± 3.9	**0.032****
IL-8 score			**0.011**
Absence (0)	5	0	
Low (1+)	1	0	
Intermediate (2+)	0	3	
$CD3^+$ per mm^2	240.9 ± 29.4	842.0 ± 314.0	**0.024***
$CD20^+$ per mm^2	204.1 ± 34.8	395.9 ± 275.4	0.999*
$FoxP3^+$ per mm^2	85.5 ± 15.4	99.6 ± 17.0	0.592**
$FoxP3^+/CD3^+$ ratio	0.35 ± 0.04	0.15 ± 0.04	**0.012****
Ki-67^+ (%)	60.1 ± 7.8 [b]	36.5 ± 3.6	**0.038****
CC-3^+ (%)	6.6 ± 2.9 [b]	7.9 ± 2.1	0.767**

Legends: ± denotes standard error; CC3: cleaved Caspase-3; MPO: myeloperoxidase; IL-8: interleukin-8; *, P values estimated by Mann-Whitney U test (*), Student-T test (**), and Chi-square test. [a], the presence of necrosis (total, neutrophil- and non-neutrophil-associated) was the only histopathological change assessed in hematoxylin & eosin. [b], Pre-treatment biopsy of P1 was not included for Ki-67 and CC-3 quantification due to low availability of neoplastic cells.



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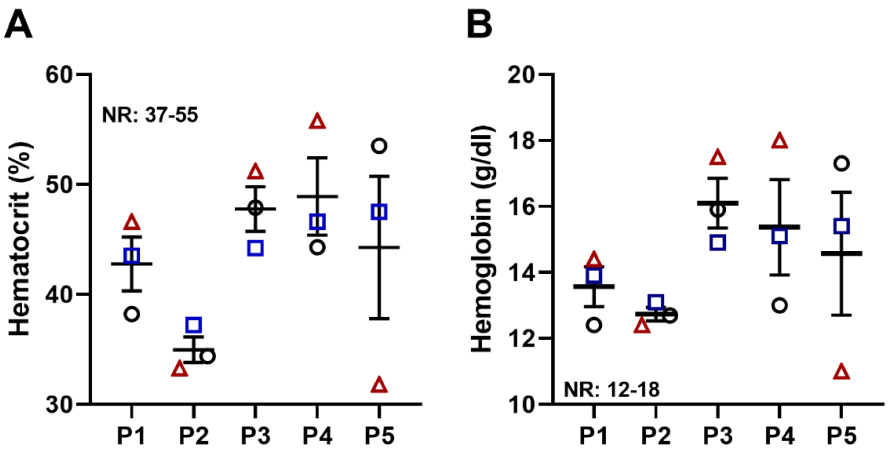

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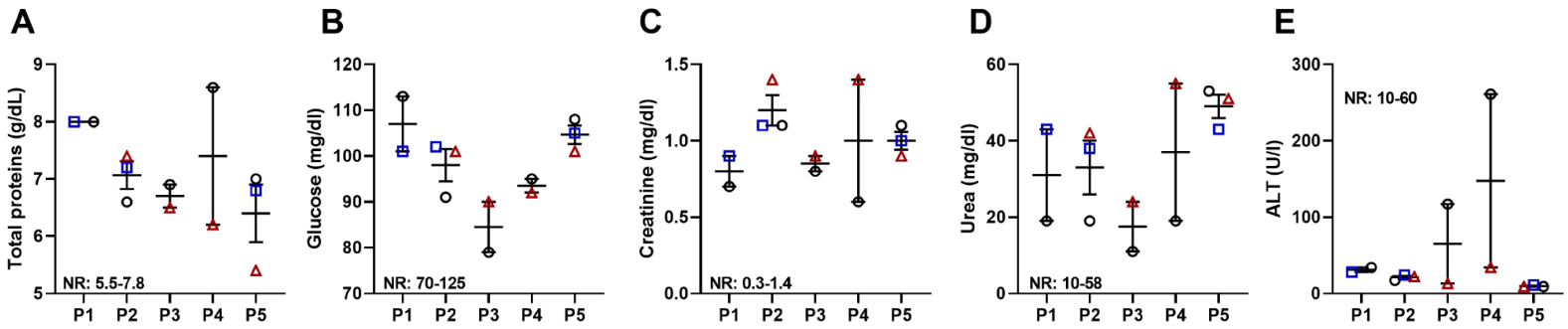

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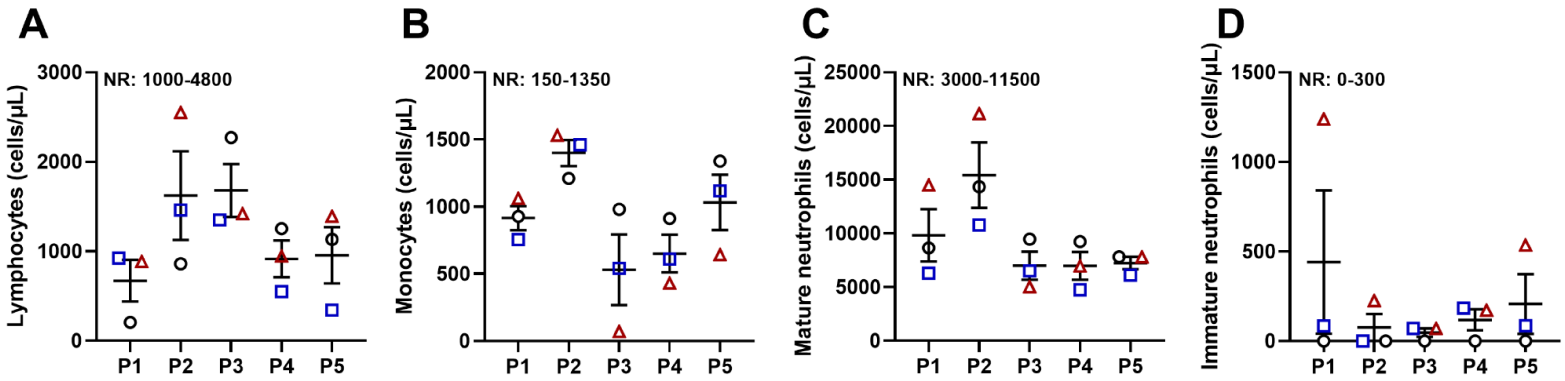

Figure S4. Neoadjuvant *in situ* eCPMV immunotherapy induced minimal changes in peripheral blood immune cells. Changes induced by eCPMV injections in lymphocytes (A), monocytes (B), mature neutrophils (C), and immature neutrophils (D). Black circles indicate basal levels at D0, blue rectangles, at D7, and brown triangles at D14. NR, refers to normal range values.

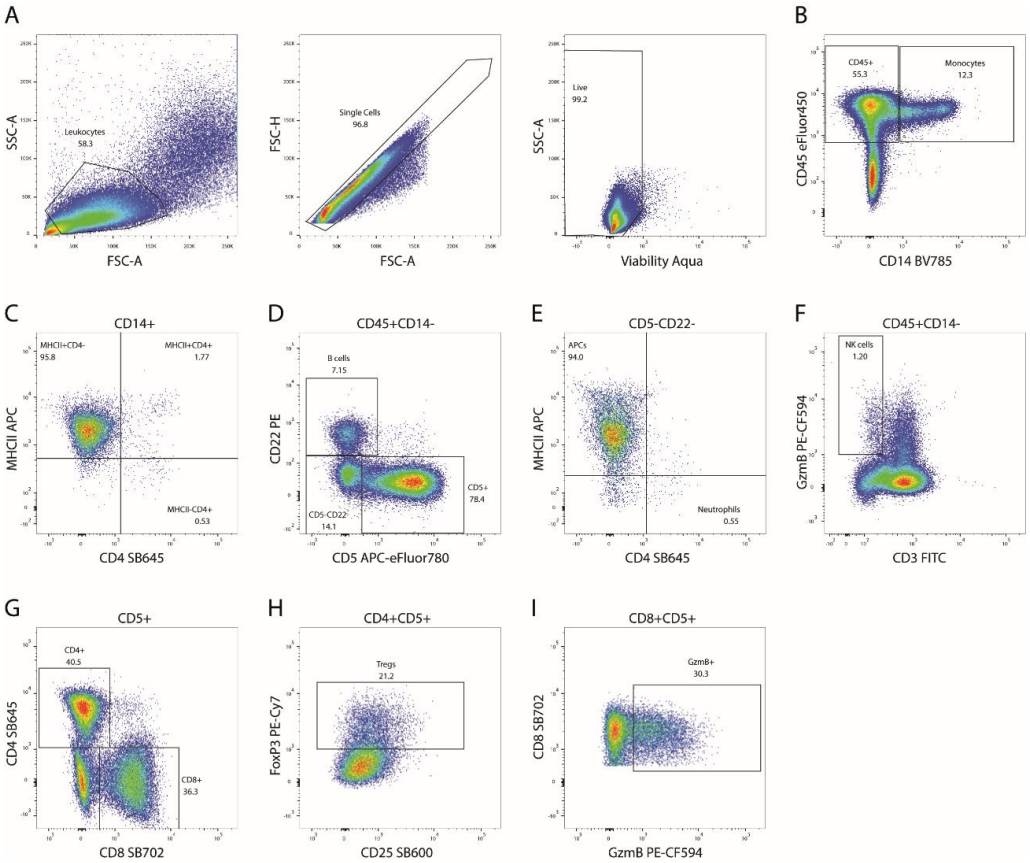

Figure S5. Gating strategy for immunophenotyping of canine PBMCs. (A) Leukocytes (SSC-A/FSC-A) were further defined as single (doublet exclusion FSC-H/FSC-A) live cells (SSC-A/Viability Aqua). (B) Live leukocytes were further discriminated in $CD45^+$ leukocytes and $CD14^+CD45^+$ monocytes. (C) Differential expression of MHCII and CD4 on monocytic population. (D) $CD45^+$ leukocytes were divided into lymphocytes ($CD22^+$ B cells and $CD5^+$ T cells) and (E) a CD22-CD5- cell population further characterized into MHCII+ antigen presenting cells and $CD4^+$ neutrophils ([8] and references therein). (F) Gating for $GzmB^+$CD3- NK cells. (G) Identification of $CD4^+$ T helper and $CD8^+$ T cytotoxic cells within the $CD5^+$ T cell population, and $FoxP3^+$ regulatory T cells differentially expressing CD25 within the $CD4^+$ T cell population (H). (I) Expression of cytotoxic cell marker GzmB within the $CD8^+$ T cell population. Parent population is indicated above the plots.

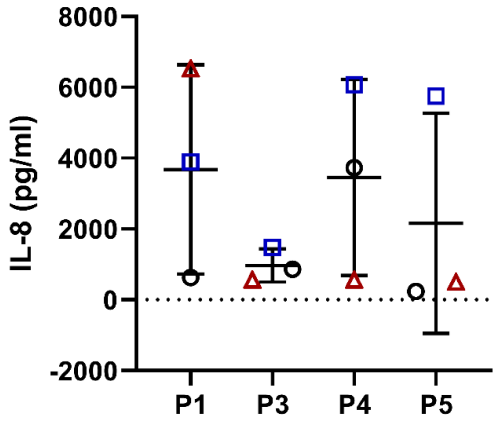

Figure S6. Neoadjuvant *in situ* eCPMV immunotherapy induced a differential effect on IL-8 in canine IMC patients. eCPMV injections induced an increase in IL-8 in P1 during the 14 day treatment, and a transient increase in P3, P4, and P5 by D7 with a subsequent decrease by D14. IL-8 levels in P1 were significantly higher at D14 compared to day 0 (P=0.039; linear regression analysis). Black circles indicate basal levels at D0, blue rectangles, at D7, and brown triangles at D14.

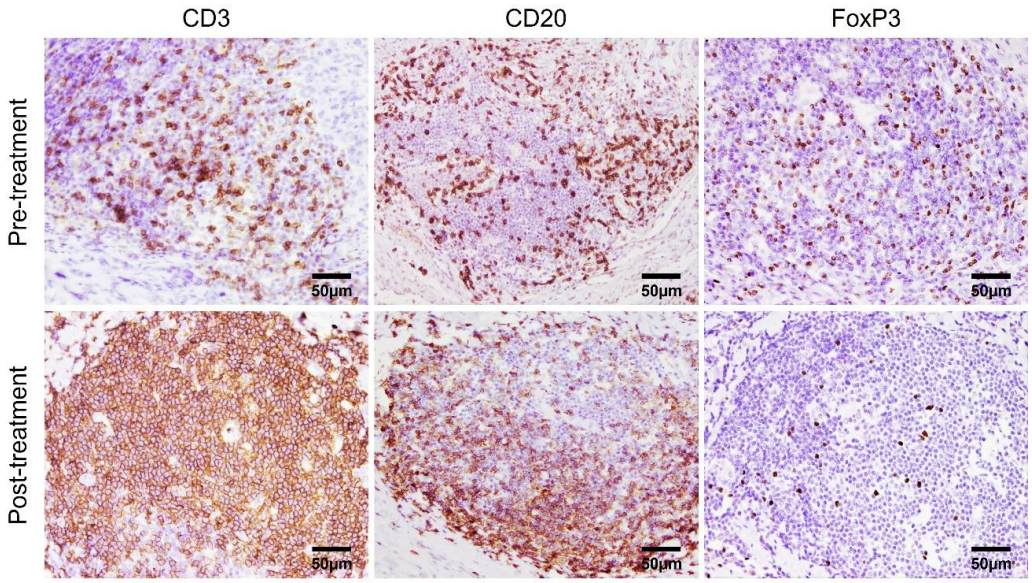

Figure S7. Neoadjuvant *in situ* eCPMV immunotherapy induces T lymphocyte infiltration and depletion of T regulatory lymphocytes in tumor samples. Representative immunostaining of tumor tissues from pre-treatment and post-treatment (P1) samples. A strong infiltration of T lymphocytes ($CD3^+$) is observed in post-treatment tumor tissue compared to pre-treatment tissues. eCPMV treatment markedly reduces T regulatory lymphocytes ($FoxP3^+$) in post-treatment tumor tissue.

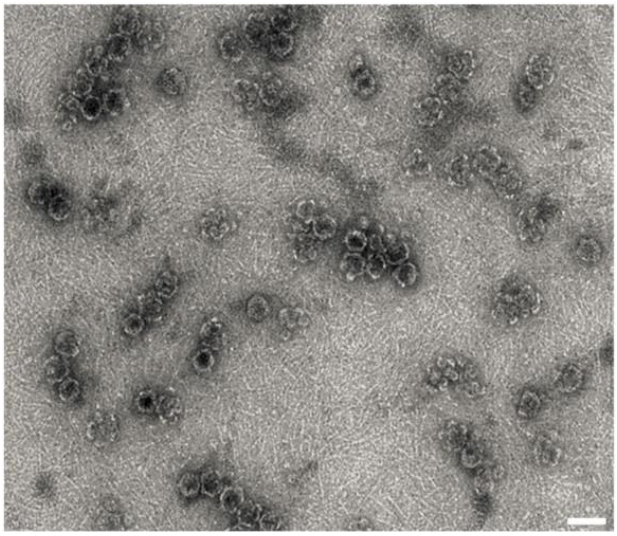

Figure S8. Transmission electron microscopy images of negatively stained eCPMV at 49,000x enlargement. White bar = 50 nm.

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Methods: Lines 87-88 & Table 1 Methods: Line 87-88 Results: Lines 164-166
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Discussion: Lines 388-389 Supplementary Data
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	No exclusions Results: Lines 164-166
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Discussion: Lines 388-389 Not applicable
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Not blinding
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Methods: Lines 154-156 Not applicable
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods: Lines 156-161, and Suppl. Data Not applicable
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Suppl. Data, Table 1 Methods: Lines 83-88, Suppl. Data, Table 1
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Methods: Lines 90-152 & Suppl. Data Methods: Lines 90-127 Methods: Lines 81-82
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Methods: Lines 90-152 Tables 1, 2, 3, S1, S3, S4, S5, S6 Not applicable

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Abstract: Lines 2-31
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Introduction: Lines 33-78 Introduction: Lines 50-65 Introduction: Lines 69-71
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Methods: Line 83
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Not applicable
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Methods: Lines 114-117 & Suppl. Data
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	Methods: Lines 145-147 Methods: Lines 91-112
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Discussion: Lines 283-374 Discussion: Lines 388-392
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Introduction: Lines 50-65 Discussion: Lines 384-387
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	Not applicable
Data access	20	Provide a statement describing if and where study data are available.	Page 2: Availability of data and material
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	Page 2: Competing interests Pages 2-3: Funding information